PROCESS OPTIMISATION FOR ACIDIFIED MILK BEVERAGE

THESIS
SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
DAIRY TECHNOLOGY

BY
CHAMPAT NARAYAN PAGOTE

DIVISION OF DAIRY TECHNOLOGY
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL (HARYANA) INDIA

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DEDICATED

IN LOVING MEMORY OF

MY DEPARTED PARENTS

AND

SUPREME - CONSCIOUSNESS

PROCESS OPTIMISATION FOR ACIDIFIED MILK BEVERAGE

By

CHAMPAT NARAYAN PAGOTE

A THESIS SUBMITTED TO THE NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY) KARNAL IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

DAIRY TECHNOLOGY

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CERTIFICATE

This is to certify that the thesis entitled "PROCESS OPTIMISATION FOR ACIDIFIED MILK BEVERAGE" submitted by Mr. CHAMPAT NARAYAN PAGOTE in partial fulfilment of the requirement for the Award of the DEGREE OF DOCTOR OF PHILOSOPHY (DAIRY TECHNOLOGY) of the National Dairy Research Institute (Deemed University) Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

Dated 13th July, 1990

(R. BALACHANDRAN)
(Guide)
Major Advisor & Chairman

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13.7.90

(CHAMPAT NARAYAN PAGOTE)

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LIST OF ABBREVIATIONS

AMB	:	Acidified milk beverage
ANOVA	:	Analysis of variance
atm	:	Atmosphere
BDI	:	Bureau of Dairy Industries
BTS	:	Body and texture scores
CAS	:	Colour and appearance scores
C.D.	:	Critical difference
C.L.R.	:	Corrected lactometer reading
CMC	:	Carboxy methyl cellulose
cP	:	Centipoise
^{o}C	:	Degree celsius
d.f.	:	Degrees of freedom
FFA	:	Free fatty acids
FLS	:	Flavour score
gm	:	Grammo
HMF	:	Hydroxy methyl furfural
H.P.H.	:	Horse power hours
IS	:	Indian Standards
kwh	:	Killo watt hour
$<$	:	Less than
$\leq$	:	Less than or equal to
µ	:	Micron
mg	:	Milligramme
µ eq/ml	:	Micro equivalent per millilitre
min	:	Minute(s)
ml/lit	:	Millilitre per litre

MPa	:	Mega pascal
M.S.S.	:	Mean sum of squares
N.D.R.I.	:	National Dairy Research Institute
nm	:	Nanometer
NPN	:	Non-protein nitrogen
OAS	:	Overall acceptability score
O.D.	:	Optical density
%	:	Per cent
psi	:	Pound per square inch
r	:	coefficient of correlation
R^2	:	Coefficient of determination
RH	:	Relative humidity
RDS	:	Reducing sugar
Rs	:	Rupees
rmp	:	Revolutions per minute
S.E.	:	Standard error
sec.	:	Second(s)
RDS	:	Reducing sugar
SNF	:	Solids-not-fat
S.S.	:	Stainless steel
TBA	:	Thio-barbituric acid
TS	:	Total solids
UHT	:	Ultra high temperature
VIS	:	Viscosity
V/V	:	Volume per volume
w/v	:	Weight per volume

CHAPTER - 1

INTRODUCTION

1.0 INTRODUCTION

In recent years there has been a great spurt world wide, in marketing new forms of flavour-enhanced liquid milk. Milk based soft drinks and beverages continue to receive a considerable amount of attention in the literature, reflecting a growing awareness of the potential of these products in the market place. Recent trends show that beverage market is one of the most lucrative in the food industry and those concerned with new product development in the dairy industry are constantly striving to develop new milk based beverages which find a ready market thereby providing yet another economic outlet for milk.

A survey in U.K. clearly showed a maintenance of diet and health concern which was having a direct impact on the sales of different types of liquid milk (Wright, 1987). A survey of the flavoured milks market indicated that sales of these products increased in the U.S.A. by nearly 20 per cent between 1970 and 1980 (White, 1983). In the countries of European Economic Community, the per capita consumption of flavoured milk during 1980 was highest in Denmark (8.4 kg), followed by Belgium/Luxembourge (6.9 kg), the Netherlands (5.7 kg) and Federal Republic of Germany (4.3 kg) (Mankowski, 1982). Considerable increase in yoghurt sale in recent years appears to have stimulated the imagination of dairy food processors in Western World in developing new forms of yoghurt and yoghurt type acidified milk based beverages. In addition to set and stirred type yoghurt, drinking types of yoghurt have become very popular. Drinking yoghurt is prepared from sweetened low fat milk using conventional yoghurt cultures. It has a suitable consistency to be drinkable at low temperature and usually has a fruit flavour. A Danish dairy is reported to be manufacturing drinking type yoghurt with 50 distinct flavours (Anon, 1986). Iya and Mathur (1986) while discussing about dairy processing industry of 21st century have predicted that during early 21st century, a large number of newer dairy products are likely to emerge having novelty of flavour, texture and structure. The use of dairy by-products in food industry is likely to increase in general.

Food packaging is another growing activity the world over, due to their numerous advantages to the industry as well as to the consumer. Aseptic packing of milk, fruit juice and milk based beverages, common elsewhere in the world, is a recent phenomenon in India (Veeraraju, 1988). The market for modified atmosphere packaging (MAP) is relatively new having emerged during the later part of 1980s. It is already forecasted that it will be the faster growing sector of food packaging over the next few years. It is now a dynamic and fast changing market, since, it offers benefits to the retailers and customers alike. The extended shelf life of product is another important aspect which is transforming the distribution chain offering the retailer greater flexibility in stock control and distribution. To more fastidious and health conscious consumer, it offers the choice of fresh chilled products with less or no additives or preservatives.

According to Prahlad (1987), in India number of soft drinks, milk based or otherwise are on incline. They are becoming popular due to reasons, such as more profit margin, convenient aseptic packs, long storage life, etc. A variety of brand names (Choco-shake, Elaichishake, Goodness, Greatshake, Frooti, Appy, Volfruit, Jiva, etc.) have proliferated, dispensing milk and fruit drinks, in popular flavours and their number is on the increase. All these drinks rely on the aseptic packaging system. Besides explaining the reasons for the popularity of these products he concluded that this development is both timely and topical keeping in step with the life style of a generation that has taken to the fast food culture.

Many of the products produced are with technical information borrowed from foreign collaboration which have lead to many problems in processing and storage of the product under Indian conditions.

The acidity or pH desired by consumers in these products vary according to the flavour desired. In most of the acidified dairy products and lactic beverages, bacterial culture is used. Several workers have considered that direct acidification may offer advantages over traditional culture methods in the continuous manufacture of acidified dairy products. The advantages may include the elimination of expensive maintenance and propagation of cultures, greater uniformity in products and better keeping qualities. Direct acidification is yet another approach to make products similar to cultured-type dairy products. It is comparatively new technique, and is not highly developed when compared with other dairy processing methods. Continuous increase in population, along with an increase in popularity of soured products are likely to combine together in an increased production of acidified dairy products in future (Rahim, 1971). Several materials are available for direct acidification. Many of them usually contain, in addition to a coagulating agent, a flavour concentrate and stabilizer. Lactic acid, citric acid, hydrochloric acid and other food grade acids have been used successfully in the manufacture of soured cream, artificial buttermilk, different cheeses, yoghurt and other miscellaneous products and beverages (Rahim, 1971; Fox, 1978).

Lactic beverages are mostly prepared using raw materials such as whey, skim milk, butter milk or whole milk with additives, such as stabilizers, colouring and flavouring agents, fruit-flavours or fruit-juices, etc. The lactic beverage is essentially prepared by using cultures. The information regarding directly acidified milk beverages, are available mostly in the patent forms. Also no published information is available about various processing parameters and physico-chemical properties of directly acidified milk based beverages.

Development of directly acidified milk based beverage will have a good scope in our country also. A systematic attempt therefore, needs to be carried out for formulating and standardizing the condition for the development of acidified milk based beverage using various ingredients. Further there is a need to make more detailed studies on the different processing parameters and their effect on the physico-chemical properties as well as on storage stability of acidified milk based beverage.

CHAPTER - 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The published information on acidified milk beverages and related products with specific reference to the composition, manufacturing technique, protein stability, and role of stabilizers and emulsifiers in low-pH milk based beverage is reviewed in this chapter. This review also deals briefly with the classification and present trends in beverage manufacture and sale.

2.1 CLASSIFICATION OF BEVERAGES

Beverages are ready-to-serve drinks prepared from food ingredients or from their by-products (viz. malt grains, fruits, honey, cheese whey, buttermilk etc.) with or without some additives (viz. colour, flavour, acidulents, stabilizers, salts, sweetners, CO_2 gas, etc.) and are consumed for various reasons, such as stimulating effect, food value, refreshing and thirst quenching quality.

Beverages, may be classified into the following major groups:

2.1.1 ALCOHOLIC BEVERAGES

2.1.1.1 Carbonated Mildly Alcoholic Beverage: e.g. Beer, Champagne, Mead.

2.1.1.2 Non-carbonated Alcoholic Beverage: e.g. Wine, Whisky and other distilled alcoholic beverages.

2.1.2 NON-ALCOHOLIC BEVERAGES

2.1.2.1 Non-alcoholic Carbonated Beverages (Soft Drinks)

a) Cola types with caramel flavourings and colourings e.g. Coca-cola, Campa-Cola

b) Ginger ale type with giner flavour.

c) Natural fruit juice based drinks.

d) Beverages with added synthetic fruit colours and flavours e.g. Orange, Lemon, Mango etc.

e) Frozen soft drinks.

2.1.2.2 Non-alcoholic, Non-carbonated Stimulating Beverages: e.g.

1. Coffee; 2. Tea; 3. Chocolate drink.

2.1.2.3 Non-alcoholic, Non-carbonated, Non-stimulating Beverages

a) Fruit or fruit juice based beverages e.g. orange juice.

b) Vegetable beverages e.g. tomato juice, carrot juice etc.

c) Meat broth beverages e.g. clam broth.

d) Protein beverages, based on soybean, fish and peanut protein concentrates.

e) Milk based lactic beverages -

Milk based lactic beverages with a few exceptions (that is alcoholic whey beverage or whey wine, and some carbonated lactic drinks) are basically cultured or directly acidified non-cultured) milk drinks with or without fruit/fruit juices and can be prepared from using whole milk (cow or buffalo milk), skim milk, butter milk, cheese whey and yoghurt.

2.2 PRESENT TRENDS IN BEVERAGE MANUFACTURE AND SALE

Since severe heat-treatment for prolonged time causes undesirable changes in the nutritive and organoleptic properties of the food, heat processing methods and techniques have been modified over the years in an effort to minimise these defects. The current trend is to use continuous processing with short time application of higher temperature. The major advantages of higher temperature short-time processing and aseptic packaging are long shelf-life and stability at ambient temperature, low energy cost for processing, improved flavour, lighter weight, lower shipping and storage costs, and other assorted benefits.

Currently many fruit juices and fruit drinks and beverages are being processed by aseptic packaging into Tetra pack or Brik pack cartons, using thickening agents such as CMC, pectin and xanthan gum (Glicksman, 1983).

Soft drink industry has grown very rapidly in India during last decade. Dodd and Gupte (1990) recently analysed carbohydrate contents and composition of some selected beverages sold in the open market. They found that in carbonated drinks, the pH generally ranged between 2.32 to 3.10. In case of fruit based soft drinks the pH ranged between 2.93 to 3.41. For milk based soft drink, the pH ranged between 5.89 to 6.39. Sucrose was identified as predominant sugar in fruit based beverages while glucose was predominant sugar in most carbonated beverages. The lactose and sucrose were identified in milk based beverages. The total sugar content of fruit based beverage was found to be higher than carbonated and milk based beverages.

With the availability of aseptic packaging system in India, fruit processing industry is undergoing rapid change. Newer developments in the area of aseptic fillers, faster and more efficient filling equipment that will equal the speed of cans and new aseptic packaging material with improved barrier properties have taken place. In view of its innumerable advantages aseptic packaging has great promise in future years. In India beginning has been made in the packaging of fruit and milk beverages, soy-products, etc. in unit packs and fruit pulps in bag-in box (Kumar, 1984).

Food packaging is a growing activity world over. In food industry, packaging plays the dominant role in marketing and in the total manufacturing activity. Food packaging costs have doubled in the last few years, but their relative costs have remained stable at about 2½ per cent of the total manufacturing cost (Veeraraju, 1988).

Newer packaging developments in this field are already bringing numerous advantages to the industry as well as to the consumer (Shenoy, 1988). Aseptic packaging of milk, fruit juice and soy-based beverages, common elsewhere in the world, are a recent phenomenon in India (Veeraraju, 1988).

Developments in packaging, particularly the introduction of 250 ml bricketttes with a straw attached to the side, have had a pronounced effect on sales. Lejeune (1984) found that the wide diversification and development of the market for flavoured milk and drinking yoghurts in France. The output of flavoured milk in France has increased from 14 million litres in 1970 to 39 million litres in 1983 with chocolate and strawberry being the most popular flavours. The output of drinking yoghurt, first marketed in France in 1974, has increased markedly but partly at the expense of conventional yoghurt sales. It is anticipated that these products, aimed primarily at children, will encourage continued consumption of dairy beverages in later life.

The output of fruit flavoured milk in 1984 in Germany was estimated at 30,000 tons, and that of kefir and traditional German soured milk at 130,000 tons (Anon, 1985a).

2.3.1.1 Acidified milk beverages Without Fruit Juice

a) Carbonated beverage:

Hawthorne et al. (1977) developed a carbonated pineapple flavoured beverage containing 16.0 per cent sugar, 0.32 per cent citric acid and 7.0 per cent milk with flavouring and colouring. The product was pasteurized or sterilized in retail containers. Addition of 0.03 per cent tri-sodium citrate before heating was found to give very good cloudiness, frothing capacity and improved taste to the product.

Ali Mohamoud and Heelsbergen (1980) claimed the preparation of carbonated milk drinks by using a mixture of milk and milk products with carbonic acid and/or savouries, sweeteners, vitamins, minerals and preserving agents with or without preservative treatment.

b) Non-carbonated beverage:

Daehler (1979) developed milk based beverage which contained 80 per cent of 3.5 per cent fat milk, 10 - 11 per cent high fructose maize solids, 0.10 per cent xanthan gum, 0.14 per cent carrageenan, 0.40 per cent carboxymethyl cellulose, plus flavouring and colouring and water to 100 per cent. The beverage was adjusted to pH 4.1 - 4.5 with food grade lactic acid or 50 per cent citric acid solution, homogenized and kept under refrigeration.

A method for manufacture of acidified milk beverage with 1.5 - 4.6 per cent milk-SNF had also been claimed, which remained stable without precipitation and separation (Anon, 1979).

Takahata (1980) prepared a stable, emulsified, acidified whole milk beverage by adding an aqueous solution of locust bean gum (0.3%) to whole milk, agitating the mixture while adding an acidifying agent at 35°-60°C to form an acidified emulsion with a pH of 3.4 - 3.6. The emulsion was further stabilized by homogenizing. The product was then sterilized and filled in suitable containers.

A stabilized acidified milk beverage was prepared by subjecting acidified skim milk with a pH of 3.35 - 3.75 and a milk-SNF content of 0.7 - 1.5 per cent to UHT treatment at 125°-160°C for not more than 10 sec. and adding sugar at a rate of 8 - 15 per cent following the heat treatment (Yasumatsu et al., 1980).

A process for acidified beverage from whole milk had been patented (Anon, 1981a). 100 gm cane sugar and 3 gm locust bean gum were dissolved with stirring in 200 ml water at 85°C. This was mixed with 400 ml whole milk. Later 1 gm citric acid, 4 ml lactic acid and 0.3 ml lemon flavour were added at about 50°C. The product was stirred for about 20 min. whilst maintaining the temperature at 50°C. More citric acid was then added to adjust the pH to 3.5, and the product then homogenized and pasteurized at 80°C. The product was claimed to be stable without curd separation.

Kim et al. (1984) reported the preparation of acid milk gel beverages from whole milk, skim milk or diluted milk with the addition of sugar, carboxymethyl cellulose and gelatin, as well as lactic or citric acid. Optimum organoleptic scores were obtained with whole milk containing 0.25 per cent each of added lactic acid and citric acid.

2.3.1.2 Milk-Acid-Fruit Juice Beverages

A British patent taken out by a Japanese firm (Anon, 1974) covered a grape juice flavoured dairy drink based on aqueous solution of sodium caseinate, citric acid and 5 per cent added grape juice.

Nishiyama (1976) reported about apple-juice flavoured milk beverage which was prepared by dissolving 200 gm of sugar in 400 ml of water, followed by heating at 80°C. After cooling to 15°C, 540 gm of cold milk, 100 gm of apple juice preparation and sterilized water to make upto 1800 ml were added. The apple juice preparation contained 4.2 - 6.0 per cent (w/v) sodium carboxymethyl cellulose, 10 - 50 per cent (w/v) fruit juice and 3.1 - 5.0 per cent (w/v) citric, lactic, malic or tartaric acid in an aqueous medium. The finished drink had a pH of about 4.4.

Tebbenhoff (1979) reported the preparation of alcoholic milk drink containing acid fruit juices, alcohol and milk containing lactic acid and/or its salts.

2.3.1.3 Acidic Milk - Fruit Juice Beverages

A beverage was prepared from natural grape juice, acid milk and sugar, and its stability was studied. The grape juice pectin content was found to be inversely proportional to the stability of the beverage. Hah (1975) reported that the pectinase treatment of the grape juice could get a stable product at pasteurization treatment of 95^{o}C for 20 min.

Arolski et al. (1977) prepared a durable fruit-dairy and vegetable-dairy drinks. The drinks were prepared by adding sugar syrup, pectin solution and fruit or vegetable mash to sour milk in that order, deaerating the mixture, homogenising under pressure at 150-200 atm., sterilizing in a plate heat exchanger at 120^{o}-125^{o}C for 5-6 seconds, followed by cooling to 95^{o}-100^{o}C. The sterilized product then bottled and the bottles sealed with crown corks before receiving further sterilization treatment at 85^{o}-90^{o}C for 8-10 min. and being cooled finally to 35^{o}C. A typical composition for fruit dairy drink had fruitmass 35.0 per cent, sour milk 36 per cent, sugar syrup 24 per cent, pectin 5 per cent and citric acid 0.1 - 0.2 per cent.

2.3.1.4 Milk-Fruit Juice Flavoured Sour Drinks or Beverages

Mc Cabe (1956) developed a fruit flavoured milk drink which was claimed that it would not curdle on pasteurization or sterilization. The process consisted of adding a special pectin preparation to the raw milk, followed by fruit juice and sugar 2-10 min. later. After holding for further 10 min, the milk was pasteurized or sterilized in the normal way.

Doesburg and Vos (1959) reported a long shelf life acidified milk drink. The best results were obtained by adding a high-ester pectin powder, mixed with sugar, to the milk over a 10 min period while stirring continuously, adding fruit juice until a pH within the range of 4.2 - 3.0 (optimum being 3.8) was reached, and waiting for at least 10 min. before pasteurizing the mixture. It should be possible to heat well-prepared mixtures at 100°C for prolonged periods. Liquid pectin preparation could also be used.

Stabilised milk-orange juice beverage was also developed in USA (Shenkenberg *et al.*, 1971). The preferred formulation contained 56.8 per cent whole milk, 38.0 per cent orange juice, 5.0 per cent sugar and 0.20 per cent carboxymethyl cellulose. The product claimed to have a shelf life equal to or better than that of ordinary homogenised milk.

Luck and Rudd (1972) demonstrated that it was possible to mix natural fruit-juices with milk without curdling, provided a suitable stabilizer is added and to make tasty, refreshing and nutritious beverages. Carboxymethyl cellulose was found to stabilize milk against curdling when heated to 95°C at pH 4.6 - 5.0 while propylene glycol alginate (PGA) stabilized milk at 95^{o}C in the pH range of 3.9 - 4.5. However, fruit juice flavoured milk was prepared using 40 per cent fruit juice and 60 per cent milk. Carboxymethyl cellulose or propylene glycol alginate at the rate 0.25 per cent (by weight) and sugar at the rate 5 - 10 per cent(by weight) was dissolved in the fruit juice. After cooling to 4^{o}C, the milk and fruit juice mixture were mixed well. Heat treatment at 120^{o}C beyond 15 min was found to coagulate the protein. The heat stability was not improved by the addition of KH_2PO_4 (Luck and Grothe, 1973).

A refreshing nutritious drink with a pH of less than 5.0 has been developed from a combination of milk and grape fruit juice in New Zealand (Proffit and Moore, 1974). The formulation consisted of 56.8 per cent (by wt) of milk, 38.0 per cent grape fruit juice, 5.0 per cent sugar and 0.2 per cent stabilizer. The processing method has been described. The product after preheating to 50^{o}C and double homogenization, was HTST pasteurized at 76^{o}C for 15 sec. before being bottled or canned.

Igoe (1976) stated that the blending of milk with acid fruit juices without causing curdling is facilitated by adding upto 4 per cent of a thickener mixture, consisting of 7 - 22 per cent carboxymethyl cellulose and 78 - 93 per cent gelatin. The fruit juice remained within the product as a finally divided, widely dispersed mixture without noticeable graininess.

Gulevia and Jain (1979) described a process for fruit flavoured beverage was with buffaloes' or cows' milk. Apple, banana and orange juices were mixed with the milk along with 6.0 per cent sugar and 0.2 per cent stabilizer.

Kern (1980) gave a procedure for the manufacture of fruit flavoured milk, in which whole or partly skimmed milk was blended with comminuted fruit flesh and/or the juice of guava or mango fruit, each of 3 - 15 per cent by volume. The risk of milk coagulation was reduced by the low acid content of the fruits used which also suppressed an excessive formation of gastric acids.

Adams (1980) has developed a carbonated strawberry apricot-flavoured milk beverage called 'soda-milk'. It is made by bubbling CO_2 through a blend of water, dried skim milk, fruit juice, flavouring, etc., then filling into bottles. It had a shelf life of upto 6 months.

2.3.2 CULTURED AND/OR ACIDIFIED MILK BEVERAGES

Mixtures of milk, fruit juice, organic acids, flavouring agents and sugar are fermented with propionic acid bacteria to yield compositions possessing unique flavour and improved preservative properties (Anon, 1970).

Soloid (1970) developed a somewhat similar beverage by a process involving heat treatment of skim milk at 90°-95°C for 40 min., addition of 5 per cent sugar, cooling to 32°C and inoculating with 2 per cent of a mixed starter culture consisting of *Lactobacillus bulgaricus* and *Streptococcus lactis* as well as lactose and sucrose-fermenting yeast. The mixture was bottled, incubated at 28°-30°C for 10-12 hours and then vigorously shaking before being stored at 8°-10°C for 24 hrs to prevent further fermentation. The beverage contained little alcohol, had a thick, creamy consistency and a sour-sweet flavour. It could also be flavoured with fruit juices.

Grozdova (197[illegible]) reported development of a product known as "Molodost", using skim milk. The process involved prolonged heat treatment of milk for 3-4 hours at 98°-100°C, cooling to 40° 42°C, incubation with a 5 per cent starter culture mixture based on 4 parts of *S. thermophilus* to 1 part of *L. bulgaricus* and bottling in 500 ml bottles. The product could be enriched with protein by the addition of dried skim milk or by concentration through evaporation, and 5 per cent sugar could also be added, the sweetened product containing 14 per cent total solids.

Pech *et al.* (1974) studied the properties of *L. bifidus* in combination with other cultures for the production of fermented milk drinks, whilst an Australian worker had studied the suitability of different milk products for whitening soft drinks (Henderson, 1974). Sweetened condensed milk in particular has been found suitable for producing a stable cloud in soft drinks with pH values between 3-6, although ring formation on the surface required further investigation.

Lev and Patkul (1979) reported development of cultured milk beverage named "Kurunga". The process consisted of clarifying milk, pasteurizing, cooling and incubating with a mixed culture including S. lactis, L. acidophilus, L. bulgaricus and Candida species, and adding the lactic cultures and yeasts separately in the ratio of 2.0 - 2.5 : 1.0. When acidity of 85^{o}-90^{o}T was reached, the product was agitated for 10-15 minutes with simultaneous aeration, after which it was cooled.

Arolski et al. (1980) gave a process for the manufacture of fruit-milk or vegetable milk beverages of long storage life, involving the mixing of 20 - 40 per cent fruit or vegetable puree with 35 - 45 per cent cultured skim milk or whole milk, as well as apple pectin, sugar syrup and 0.1 - 0.2 per cent citric acid, the mixture being heated and sterilized.

According to a Japanese patent (Anon, 1980a) a fermented milk beverage was prepared by culturing a Lactobacillus spp in a medium containing no casein, followed by mixing the fermentation medium with milk protein, sugar and acid. Protein precipitation during the fermentation stage was claimed to be avoided by adopting this procedure.

Mackenzie *et al.* (1985) reported cultured egg/milk product consisting of 15 per cent liquid whole egg, 6 per cent dried skim milk, 4.0 per cent sugar, 0.2 per cent stabilizer and 74.8 per cent milk (3% fat). It was made by dissolving the ingredients (except the egg) in the milk at 50°C, homogenizing the mixture, heating to about 85°C for more than 30 minutes, cooling to 45°C, with the egg being stirred in preferably at below 70°C. Special culture of *L. bulgaricus* and *S. thermophilus* were then added, the mixture incubated and the product cooled to 4°C. The product was intended as an alternative to yoghurt.

Vrdoljak and Krsev (1983) described the development of a fermented permeate from ultra-filtered milk. The permeate obtained from 2.1 - 2.2 per cent fat milk, averaged pH 6.35, lactose 4.06 per cent, protein 0.32 per cent, fat 0.03 per cent and minerals 0.37 per cent. Permeate was heated to 100°C, decanted carefully, cooled and incubated with 2-3 per cent lactic acid culture. Following the addition of various flavouring agents including rum, coffee, blackberry, pineapple or citrus, it was used successfully as a base for preparation of pleasant beverages. It was reported that those fermented with yoghurt culture produced the best product.

Prakoppova *et al.* (1984) investigated the possibility of utilizing a streptococcal culture (ZS 25) isolated from ewes' milk lump cheese, in the production of cultured beverage. The culture was added at the rate of 10 ml/litre (with or without *Lactobacillus bulgaricus*) to homogenised, pasteurized milk cooled to 30°-35°C. Optimum organoleptic scores for the resulting beverage were obtained with the addition of 0.2 ml of *L.bulgaricus* culture per litre and incubation at 30°C for 16-18 hours. This beverage had a keeping quality of upto 7 days at 10°C.

A carbonated, fermented milk beverage was produced (Evers, 1984) by incubating a severly pasteurized homogenized low fat, sweetened milk at 30°C with 1ST yoghurt culture to pH 4.2. The mixture was then cooled to 15°C and the pH adjusted to 4.1 with citric acid. After blending in water containing dissolved citrus pectin and orange essence, the product was homogenized, tempered, re-homogenized, heated to 90°C with a 30 second holding time, cooled, carbonated at 1.2 bar and packaged in gas-tight containers. The product was reported to remain stable for at least 6 months.

New type of cultured milk products with citrus fruit flavouring had been reported from the USSR (Zobkova *et al.*, 1985). This beverage was made essentially from a mix consisting of (per 1000 kg) 319 kg of 3.2 per cent fat milk, 531 kg skim milk, 99.9 kg mandarin syrup, 0.1 kg citrus essence and 50 kg skim milk culture (*lactic streptococci*).

2.3.2.1 Concentrated and Dried Cultured Milk Beverage

Siscar et al. (1985) reported a sweetened acidified milk concentrate intended for dilution as a milk beverage. The product was made in four flavours and was based on milk cultured with L.bulgaricus, blended with sugar in different proportions. Sensory evaluation tests gave optimum results for a 1:1 mixture of cultured milk and sugar, with 0.5-1.0 per cent added lemon flavour concentrate. Samples stored at room temperature (26°-33° C) exhibited browning and protein settling after 6 weeks, whereas refrigerated samples (4°-5°C) gave no indication of physical changes after 12 weeks. Storage stability was maintained by the simultaneous determination of total soluble solids, total titratable acidity, per cent lactic acid, pH and total plate count.

A sterilized cultured milk beverage was developed by Murakami et al. (1978). The process included the mechanical stirring of sterilized sweetened fermented milk (40-45% sugar) to reduce its viscosity. The resultant product was free from stabilizers and was used for preparing beverages by dilution with ordinary or carbonated water.

Nakagawa (1981) patented a process for preparing beverage by fermenting wort with lactic acid bacteria. The wort was fermented with L. casei at 35°-37°C for about 3 days before being concentrated and spray dried. For consumption purposes, it was dissolved in fresh or reconstituted milk without causing coagulation, producing a nourishing milk.

2.3.2.2 Cultured and Acidified Skim Milk Beverage

Interest was also shown in the development of different types of cultured or acidified beverages. Anon (1981a) reported a process which involved pasteurization of skim milk followed by cooling to 37°C. The cooled milk was then inoculated with L. bulgaricus and incubated to pH 3.30. About 10 kg of fermented product was then mixed with 60 kg water and pH was adjusted to 3.3 by lactic acid to obtain a product containing 1.2 per cent total solids. Following pasteurization at 90°C, the pH was readjusted to 3.70 by the addition of sodium citrate solution, and desired amount of sucrose and lemon flavour was added. The mixture was repasteurized at 90°C. It was claimed that the drink, even following storage for 6 months at room temperature had a dense milky white colour and a stable protein dispersion, and did not require the use of stabilizers.

2.3.3 BUTTER MILK BASED ACIDIC BEVERAGES

Butter milk forms the base for a number of new type of beverages reported in the literature.

A pasteurised butter milk drink with 14 days storage life under refrigerated condition was made using lemon syrup, glucose and a thickening agent (Hansen, 1970).

Zschevge (1971) reported that the freshness of cultured butter milk can be maintained over long periods by adding before or after acidification, a mixture consisting of a sugar alcohol of higher valency and pectin. Preferably 2 litres of mixture consisting of 60 parts sugar-alcohol and 40 parts pectin were added to 1000 litres of butter milk, the mixture being heated to 85°C for 15-20 minutes. Pure sorbitol was the preferred sugar-alcohol and polygalacturonic and methylester the preferred pectin.

A beverage was made by standardizing fresh buttermilk with cream to 1.0 per cent fat, adding 2.0 per cent dried skim milk (previously reconstituted in a portion of butter milk) to obtain a total solids content of 11.5 per cent and pasteurizing the product at 85°-87°C, preferably with homogenization at 9.8-14.7 MPa. It was then inoculated with suitable lactic culture and incubated for 12-16 hours at 22°-25°C to an acidity of 70°-90°T; and then packed. The product contained more than 1 per cent fat and 10.5 per cent solids-not-fat, less than 150°T acidity with a clean refreshing lactic flavour, uniform consistency and exhibited no whey separation (Barzhinskas and Urbene, 1977).

A butter milk-whey beverage named 'Sudam' was developed in India (Waghmare *et al.* 1978). Firmly set whole milk or skim milk coagulum with about 1 per cent acidity was diluted with an equal amount of whey and the mixture churned. The resultant butter milk whey mixture was clarified by boiling and removal of the precipitate was done by decanting or straining. Optimum acidity of the clarified product was 0.4 - 0.6 per cent. Water was added to reduce the acidity as required and 12-14 per cent sugar added, plus 0.1 - 0.2 per cent gelatin, if desired. The beverage was cooled after boiling and aged for at least 48-72 hours at refrigeration temperature. The average composition of the product was reported to contain 14.43 per cent total solids, 1.75 per cent lactose, 0.43 per cent protein and 0.09 per cent fat.

Gushchina et al. (1979) reported a process for preparation of butter milk beverage. It consisted of concentrating fresh butter milk to 15 per cent total solids, standardizing to 1.5 per cent butter fat by the addition of milk, pasteurizing, cooling to 25°-28°C, and incubating with a mixture of *Str. lactis*, *Str. cremoris*, and *Str. acetoinicus* to form a firm coagulum with an acidity of 75°-85°T. After stirring the coagulum, cooling to 10°-15°C and adding fruit syrup, the product was stirred vigorously to a uniform consistency, packaged and stored at 8°C. The product had a creamy consistency and a clean lactic flavour.

Another lemon-flavoured butter milk drink possessing excellent keeping quality was prepared by mixing acid butter milk with lemon concentrate, glucose and cream, and packaged in 500 ml cartons (Vemling, 1980).

The preparation of a base for enriching butter milk beverages was patented by Zharenov and Eremin (1981). This involved mixing 47 - 49 per cent of butter milk with 35 - 45 per cent of vegetable protein and 0.1 to 0.3 per cent dry egg yolk. Following stirring and the addition of lactic culture and pepsin, the mixture was homogenized and ripened at 16°-18°C for 36 - 48 hours. The base, with an acidity of approximately 45°T was added to fresh butter milk which was then pasteurized at 85°C for 5 minute and cultured with a mixed L.acidophilus/ .Str. lactis starter in a 20:80 ratio

2.3.4 WHEY BASED SOUR DRINKS/BEVERAGES

The use of whey in beverage preparation continues to attract interest from different parts of the world. Whey beverages of various types have been gaining popularity for some time and a number of interesting reports have appeared in the recent literature.

2.3.4.1 Acidified Whey Drinks

Basso et al. (1973) had given a formula for flavoured beverage mix containing 16 gm of dry egg albumin, 16 gm dry whey solids, 24 gm sucrose, 1.1 gm citric acid, 0.013 gm orange colour and 0.6 gm orange flavour. The ingredients were dissolved in 235 ml water to yield a beverage of pleasant flavour and aroma.

Schuster et al. (1977) invented a refreshing beverage with the appearance of lemonade. The product consisted of 70-80 per cent whey, less than 5 per cent each of lactic acid, yoghurt and lactose, as well as milk protein. The beverage had a pH of 4.2, and also contained fruit juice and fruit pulp. It was further reported that it could be stored for over 6 weeks at 20°C or for 2 months at 8°C without requiring the addition of preservatives or high temperature treatment.

Nelson et al. (1972) reported the development of fruit flavoured drinks containing 75-90 per cent of untreated whey. Amongst suitable flavouring, peach puree (20%), strawberry (10%), and red raspberry (10%), orange flavours have been used successfully. It was reported that synthetic red raspberry flavour gave better results than some of the other synthetic flavours tried. The drinks usually adjusted to 18°-20°C Brix had a pH of approximately 3.6, the desired pH adjustment done with citric acid solution.

Iverson (1984) reported a method for production of a whey-fruit drink involving addition of fruit concentrate, sugar, flavouring and acidifying agent (to bring the pH to 3.7-3.8) and a stabilizer (mexpectin RS 450) to whey. The mixture was pasteurized at 75°-85°C, homogenized at 200 kg/cm^2 cooled to 20°C and packaged. It was suggested that such products could be marketed as fruit juice milk with protein to improve consumer appeal.

D'Yachenko and Sqares (1984) claimed success in the manufacture of an orange-flavoured whey beverage. A typical formula for production of one ton of fruit-whey beverage was also given.

2.3.4.2 Whey-Fruit Juice Beverages

Anon (1969) reported that the acid whey powder (4-6%) was combined successfully with orange juice to make an acceptable and nutritious drink.

Anon (1973a,b) reported a beverage that could be produced by combining orange juice with a stabilizer and cheese whey concentrate. The beverage which was similar in flavour and appearance to orange juice, had a protein content close to that of milk.

According to Wiesenberger et al. (1983), a whey drink could be prepared from a mixture of 50-65 per cent apple-juice, 3-5 per cent passion-fruit juice, 10-20 per cent white grape juice, 10-20 per cent lemon juice and 11-17 per cent of a liquid whey protein concentrate containing hydrolysed lactose, flavour substances, minerals and vitamins, with a pH of 4.3. The beverage after pasteurization at 75°-90°C was packaged hot, and after holding for 5-20 minutes was allowed to cool.

Niketic et al. (1984) prepared an homogenized and sterilized at (95°C/5 sec) soft drinks from acid or sweet cheese whey, using different types of stabilizer and fruit concentrates such as mandarine, orange, lemon or apple. The product was reported to contein 11 per cent total solids, 0.8 per cent protein, 4.7 per cent lactose, 0.76 per cent minerals, 0.0086 per cent ascorbic acid and essential amino acids.

2.3.4.5 Cultured-whey Drinks or Beverages

a) Flavoured whey drinks:

Grinene and Prantskyavishyus (1977) described a method for obtaining a soured milk beverage from whey. It involved the addition of sugar and aromatic substances to whey, the mixture being pasteurized and cooled, an inoculum was then added. After souring, the product was stirred, cooled and saturated with carbon dioxide. The inoculum essentially consisted a mixture of Str. cremoris and and Str. diacetilactis added at a rate of 1.0 - 1.2 per cent. Vanillin and cinnamon extract were added preferably after cooling the soured mixture.

A flavoured whey beverage, reported by Marhounova and Mergel (1980) was made by mixing filtered whey with a stabilizer, followed by pasteurization at 80°C, homogenization, incubation at 40°C with a 2 per cent Lactobacillus starter for 15 hours to pH 3.5; addition of 5 per cent sugar, aroma or fruit concentrate, repasteurization and homogenization, packaging in glass bottles, at 62°-66°C and rapid cooling. The beverage contained 13 per cent total solids and had a pH of 3.5 - 3.7 and it was claimed that the product had a keeping quality of 3-6 months.

Srivastava and Lohini (1986) described a method for preparation of an acidified whey drink 'Parag', by blending clarified paneer whey and butter milk in equal parts, heating to 100°C for 15-20 min., cooling and incubating with L.acidophilus plus Str.thermophilus at $39^{o}C$ for 20-25 hours, clarifying, adding 10-12 per cent sugar syrup, pineapple flavouring, colourant and finally pasteurizing the mixture.

✓ Jayaprakasha et al. (1986) suggested a method for whey beverage using deproteinized and clarified whey from cheese, chhana or acid casein. The process consisted of adding 6-12 per cent sugar, 0.02-0.4 per cent citric acid, and flavourings at 0.15-0.45 ml per litre of whey, followed by in-bottle pasteurization or sterilization with or without carbonation. Optimum sugar level was found to be 10 per cent and orange-flavoured drinks had the highest scores. Pasteurized carbonated drinks proved the most acceptable.

b) Fruit flavoured whey beverages:

Vieira et al. (1985) reported the preparation of acidified beverages based on ultrafiltered cheese whey (9.6% total solids, 4.2% protein) incubated with yoghurt starter and with the addition of 3-7 per cent fruit concentrates (orange, lemon, passion fruit or cashew concentrate), 7.0 per cent sucrose and 0.5 per cent stabilizer, followed by heating at $85^{o}C$, homogenization and then packaging.

c) Natural whey drinks:

Bovarian dairy factory introduced low-calorie whey product enriched with natural vitamin E, named 'Big M' (Anon, 1983). Renner (1983) had given the details of the manufacture of 'Big M'. The process involved the acidification of whey with L.acidophilus culture, addition of 70 mg ascorbic acid and 200 mg tocopherol per litre, and UHT-sterilization at 135°C for a few seconds. Minimum 4 months storage life of the product was reported at room temperature. Yet another method for making whey drink had been suggested by Vieco de Velez (1986) which consisted of: (i) concentrating whey five-fold by ultrafiltration, (ii) incubating the retentate with 1-5 per cent of a lactic acid culture at 35°-50°C for 8-18 hours, (iii) adding stabilizers and pasteurized cream with or without fruit concentrates, preservatives, or sugar, (iv) heating to partially denature the whey proteins, and (v) homogenizing either before or after the heat treatment. A shelf life of upto 45 days at 6°C was claimed for the beverage.

Tuohy et al. (1988) prepared the beverages by blending either acid- or sweet-whey fermented with lactic acid bacteria, with or without orange juice or apple juice, sweetening with sucrose and pasteurizing at 85°-95°C for 15-40 seconds to destroy pectinesterases. It was claimed that 86 per cent of consumers rated this product acceptable to extremely good.

2.3.5 YOGHURT BEVERAGES OR DRINKING YOGHURTS

Consumer preference survey for yoghurt drinks carried out in the USA (White et al., 1984) had confirmed that fruit flavoured drinking yoghurt would be acceptable to a major segment of the consumer market (especially those in the 19-40 years of age group), and the products should also gain in popularity as consumers become increasingly aware of the nutritional importance of such foods.

The yoghurt in liquid forms has a thirst quenching function. A smooth, slightly viscous texture guarantees this effect. In this case, all the standard fruit flavours and tropical and subtropical and cocktail combinations could be used (Mann, 1985).

Of all the recently introduced new types of yoghurts, pride of place must go to drinking yoghurt which have had a good reception in a number of different countries. Drinking yoghurt are among the potentially most exciting dairy products developed during recent years and this had reflected in the literature (Mann, 1987).

2.3.5.1 Acidified Yoghurt Drinks

Aroiski et al. (1978) prepared high quality soured milk beverage with improved shelf life by mixing 35-54 per cent yoghurt with sugar syrup, apple pectin, 20-40 per cent natural fruit or vegetable puree and citric acid. The mixture was homogenized, sterilized at 120^{o}-$130^{o}C$ for 50-70 seconds, then cooled, packaged and additionally sterilized.

Hidalgo and Dalan (1983) reported the preparation of a drink with yoghurt flavour. This process included enzymatic hydrolysis of milk proteins in aqueous suspension, addition of lactose to suspension, and fermentation of suspension with yoghurt culture. If necessary, additional edible grade acid could be added to maintain the desired acidity. The insoluble fraction was then separated and the drink was packaged and pasteurized. The product had a mouthfeel similar to that of milk and was claimed, both to be nutritive and refreshing.

2.3.5.2 Long-life Drinking Yoghurt or Beverages

a) Fruit yoghurt drinks:

In Denmark, a new yoghurt drink with a total solids content of 16 per cent and a shelf life of more than 6 months at room temperature has been developed (Anon, 1983a). The product contained skim milk yoghurt, fruit juice and a new pectin stabilizer (Mexpectin RS 450) which stabilized the protein during heat treatment carried out after incubation.

Anon (1983b) reported two long-life drinking yoghurts in Germany. The first one, named 'Kuribic', had orange, passion fruit, pineapple and kiwifruit flavour, whilst the second one was further fortified with multi-vitamins, besides fruit flavours. Both types had sugar, stabilizer and flavouring agents, and were packaged aseptically in 0.5 litre Tetra Brick cartons with drinking straws attached.

b) Flavoured yoghurt drinks:

A Dutch company had also patented a process for drinking yoghurt (Evers, 1983). Yoghurt prepared conventionally to a pH of 4.1-4.2 was mixed with fixed quantities of water, sugar, pectins and fruit flavours. After homogenization, the mixture was heated to 45^{o}-55^{o}C under agitation, a temperature differential of less than 10^{o}C being maintained between the heating medium and the product. After cooling to 25^{o}-30^{o}C, the mixture was rehomogenized, pasteurized or sterilized, cooled and packaged aseptically; a keeping quality of 6 months at room temperature was claimed for this drinking yoghurt.

New drinking yoghurt produced by a Canadian company is called 'Yourtino' (Anon, 1985a) containing 1.5 per cent fat and was available in strawberry, raspberry and peach flavour and packed aseptically with a shelf life of about six months.

c) Natural/Plain yoghurt:

Milk with 1 per cent fat was UHT-sterilized at 135^{o}-140^{o}C combined with simultaneous homogenization after preheating, at 100 atmosphere and after sterilization at 40 atmosphere. The heated milk was cooled to the incubation temperature (40^{o}-50^{o}C), inoculated with 4 per cent yoghurt starter, incubated until pH 4.2 was reached, followed by cooling to below 10^{o}C and adding citric acid to adjust pH to 4.1. After that yoghurt was pasteurized at 75°C and homogenized at the same pressure as that was used during sterilization of the milk (Schulz, 1969).

Hermann (1980) reported a process involving pasteurization and homogenization of an aqueous base mix consisting of 4-14 per cent dried skim milk, 0.5-4 per cent fat and sugar, inoculation with a yoghurt culture, and incubation to provide a coagulum with a titratable acidity of 0.75-1.25 per cent. The coagulum was broken by agitation to form liquid yoghurt, followed by addition of 0.15-0.40 per cent of a stabilizing composition containing 60-75 per cent of Xanthan gum and 25-40 per cent guar gum. The stabilized liquid yoghurt was pasteurized and homogenized to stop further fermentation and to provide a product of extended shelf-life and reduced tendency to separate.

2.3.5.3 Instant Yoghurt Drink Mix

An instant yoghurt drink mix with natural banana, strawberry and tropical fruit flavours, but without any added preservatives, had been launched in the Western USA. It was reconstituted into a drink with milk or butter milk and was claimed to be creamy, refreshing and to possess a natural yoghurt flavour (Anon, 1982).

An instant dried yoghurt for reconstitution in water and a dried yoghurt/carrot powder for reconstitution in milk to produce a drinking yoghurt was developed in Israel (Freeman, 1985).

2.3.6 CONCENTRATED AND POWDERED ACIDIFIED MILK BEVERAGES

The aim of producing concentrated or dry beverage mix is that which can be reconstituted quickly and simply to give a stable acidified beverage.

2.3.6.1 Concentrated Beverages

According to a Japanese patent (Anon, 1975), concentrated orange juice could be combined with fermented milk, sugar, and water to yield a beverage which would be stable against coagulation.

Inagami and Tanaka (1976) prepared a syrup for soft drink from cows' milk, sugar and edible acid to give a final concentration of 3-5 per cent milk SNF and upto 15 per cent of sugar in the syrup with sufficient edible acid to obtain a pH of 3.0-3.4. The syrup when diluted with water to 3-4 times its original volume, produced a soft drink with a stable, white turbidity.

Tucker and Moss (1974) had reported on the use of pilot plant equipment for the freeze concentration of milk, whey and milk-fruit beverages. Optimum efficiency was obtained with a 2-stage treatment, removing approximately half the water content at each stage.

2.3.6.2 Beverage Powder

Bauchau (1972) recommended that the milk-durian fruits beverage powder containing 16.6 per cent milk solids gave an acceptable and well-homogenized product.

Luck and Hopkins (1974) had developed a fruit juice flavoured milk powder. It was found that mixtures of milk powder and dehydrated fruit juice could be reconstituted without curdling provided that a suitable stabilizer had been added. The stabilizing effect on casein seemed to be greater when the stabilizer was dissolved in the milk and then dried, than when it was dry mixed with the milk powder. After reconstitution, a dry mixture of milk powder and sodium-carboxymethyl cellulose (CMC) was stable at pH 4.5-5.0, propylene glycol alginate (PGA) producing a stabilization in the range of pH 3.9-4.9. Water temperature at reconstitution had little effect on the stabilizing ability of CMC but had a pronounced one on that of PGA (minimum stability between 30°C and 60°C).

Sirett *et al.* (1978) developed dry beverage mix composition and process thereof. Carboxymethyl cellulose (1.5 parts) was dispersed into 220 parts of chilled whole milk, the mixture was spray dried and then dry-blended with sucrose, citric acid, flavour and colour. On reconstitution with water in a ratio of 1:4 a tangy beverage with a desired flavour and texture was obtained, and coagulation or curdling of the milk on storage was not observed.

Trop (1980) reported a powdered composition which was suitable for mixing with milk to obtain an acidified beverage. It comprised (per litre liquid milk) of about 10-50 gm of an acidogen and about 2.5-20 gm of a mild acidic buffer. The acidogen was preferably selected from the group consisting of lactones and anhydrides. Fruit flavour (strawberry, pineapple, apricot), yoghurt flavour, and dried cells of lactic acid bacteria could be incorporated.

Sirett et al. (1981) patented a typical dry mix containing 50.0 per cent dried whole milk, 43.72 per cent sucrose, 3.58 per cent citric acid, 2.6% per cent carboxymethyl cellulose with required amount of flavouring and colouring agents. The mix could be reconstituted with milk or water to produce an acidified milk drink without any curdling.

Dried beverage mix of the type suitable for mixing with milk or water to produce a drink, had also been discussed with reference to definitions and relevant legislation in Federal Republic of Germany (Benk, 1982).

Trop (1985) developed another powdered composition that upon mixing with milk, formed an acidified milk drink without curdling of the milk proteins. The composition consisted of 1-9 per cent edible acid, 0.5-1.0 per cent instant cold-water soluble gelatin, 1-10 per cent edible natural or modified polysaccharide gum, 20-90 per cent sweetening agents and flavouring and colouring agents. Another mix that when mixed with water formed an acidified drink without curdling of casein, included 5-6 per cent of casein. The ratio of gum to gelatin in both composition was between 2:1 and 10:1 to assure the formation of a non-gelled liquid milk products.

High technology has been applied by Egyptian workers (El-Shibiny et al., 1985) to produce a skim milk/ strawberry powder for reconstitution into a strawberry flavoured milk beverage. Concentrated permeate (27% total solids) prepared by ultrafiltration of reconstituted dried skim milk followed by reverse osmosis and vacuum evaporation, was mixed in a 3:1 ratio with a homogenate of whole strawberry fruits (9.0% total solids). The mixture was spray-dried after straining off the supernatant and dry blended in equal parts with cane sugar and citric acid was added at the rate of 2 per cent. The reconstituted beverage (15% total solids) was assessed as organoleptically acceptable.

El-Salam et al. (1986) reported work on the use of skim milk permeate in the preparation of spray-dried beverages. Permeate obtained by ultrafiltration of reconstituted skim milk (10% total solids) was concentrated by reverse osmosis and evaporated under vacuum to 27 per cent total solids. Concentrated orange juice (18% total solids) was blended with the skim milk permeate in 1:3 ratio. After adding artificial colour, the mixture was spray-dried, sugar was incorporated to the resultant powder by dry mixing. During storage in polyethylene bags for 6 months at room temperature, the composition was reported to have remained almost unchanged - apart from variable losses in vitamin contents.

2.3.7. MILK SHAKE TYPE BEVERAGES

Katagiri and Yamamoto (1982) patented a process for the manufacture of a low-fat instant milk shake with good foaming properties. This involved mixing dried skim milk with sweeteners, foaming agent, gelling agent, flavouring, emulsifier, colouring etc.

Baudach (1983) suggested a formula for preparing a dry mix for making milk shakes. It consisted of 250 gm spray-dried whole or skim milk, 160 gm sugar, 85 gm whipping agent, 3.64 gm thickener, 1 gm sodium caseinate, 0.06 gm artificial yellow colouring agent and 0.30 gm vanilla flavour or a number of alternative flavours. Reconstitution could be done with water, liquid milk or evaporated milk. This shake was reported to give a volume increase of 300-400 per cent with a product volume retention of upto 3 days.

Kahn and Lynch (1983) reported a milk shake which maintained a stable volume when stored at vacuum to 27 per cent total solids. Concentrated orange juice (13% total solids) was blended with the skim milk permeate in 1:3 ratio. After adding artificial colour, the mixture was spray-dried, sugar was incorporated to the resultant powder by dry mixing. During storage in polyethylene bags for 6 months at room temperature, the composition was reported to have remained almost unchanged - apart from variable losses in vitamin contents.

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Barbier et al. (1984) and Barbier and Rialland (1986) reported a process for the manufacture of long life beverage without addition of stabilizers. About 1000 litres of cheese whey with pH 6.35, clarified centrifugally and cooled to 10°C, was allowed to percolate through a bed of 40 litres of a weakly acid cation resin (Dowex CCR-2). 75 kg sucrose and 0.8 kg apple aroma were then added to the acidified whey, the mixture then was preheated to 85°C, sterilized by an indirect method at 135°C for 2-5 seconds, and packaged aseptically after cooling. The end product was claimed to be stable besides possessing a mild, pleasant flavour. However, it was reported to contain considerably less calcium and potassium than the initial whey.

Efstathiou et al. (1987) described a method for manufacturing a low-acid milk-juice beverage. The milk component was first treated with a cation exchange resin to give a pH of 3.2 - 4.5, followed by an anion exchange resin to give a pH of 3.5 - 4.5. The juice component was similarly decationized to pH 2.5 - 1.3, deanionized to pH 8 - 11.5, and then acidulated by cation exchange to pH 3.0 - 4.5. Blends of the treated juice and milk were then homogenized and pasteurized or sterilized. It was claimed that the product had good stability with minimal curdling or precipitation, and it contained no stabilizers.

2.4 PROTEIN STABILITY

Traditionally, the acidulation of milk is done by fermentation under controlled conditions to produce yoghurt, cultured drinks and related products but systems are now available to produce sour milk drinks without microbiological processes.

2.4.1 BUFFERING SALTS

It is sometimes desirable to treat milk in such a way as to alter its ionic balance in order to stabilize it or otherwise improve its utility for a particular purpose. The best example of such a treatment is the addition of phosphate or citrate to stabilize milk against subsequent heat coagulation. Additions of phosphate or citrate result in the binding of more of the calcium in the form of soluble complexes and decreasing the activity of the calcium ions (Jenness and Patton, 1959). Decreasing ionic strength, as by dilution of the drink with water, impairs stability, stability of such diluted drink can be restored by addition of buffer salts (Glahn, 1982).

2.4.2 HYDROCOLLOIDS

The protective hydrocolloids allow the pH to drop below the isoelectric point of casein without casein precipitation. For example, high methoxylated pectin exhibited a protective hydrocolloid effect in low-pH milk products (e.g. in yoghurt). The reaction mechanisms has been explained as follows.

At the inherent pH of milk, the casein micelles are observed in uniform colloidal suspension. The net negative electrostatic charge displayed by the micelles effects repulsion between them. As the pH decrease, approaching the isoelectric point of casein (pH 4.6), the total charge balance is equalized, repulsion is diminished, resulting in the development of micellar aggregates, essentially coagulating and setting of the yoghurt. Acidity development will continue and the pH is reduced below the isoelectric point to say 4.1, at this point the casein micelles will be positively charged.

To maintain the charge repulsion, high methoxylated pectins can be utilised. At low pH values the pectin molecule will be negatively charged and interaction with the positively charged casein micelles occur to form a stable complex. This promotes the appropriate charge balance between adjacent micelles, preventing them from agglomerating, thus avoiding sedimentation and precipitation (Gregory, 1985).

2.4.3 SOLUBLE PROTEINS

2.4.3.1 Preparation of Acid Soluble Casein

Spasova and Kozhev (1979) described the investigation carried out to develop a method of producing a modified milk protein, soluble in an acid medium, using HCl-casein and low-Ca precipitate (obtained by precipitation of skim milk with heat, acid or $CaCl_2$). Good results were obtained by lowering the pH of 10 per cent solution of HCl casein below the isoelectric point in order to convert the casein to cationic form. Addition of 10 per cent solution of citric or lactic acids increased casein solubility to over 80 per cent, the pH being reduced 2.4 with citric acid and 2.0 with lactic acid. Further increase in solubility was obtained following mechanical treatment in a colloid mill. The acid-soluble milk protein is intended for use in carbonated soft drinks, as well as in fruit/vegetable based baby foods.

2.4.3.2 Preparation of Soluble Caseinates

Caseinates are manufactured by adding an alkali (NaOH, KOH, $Mg(OH)_2$, $Ca(OH)_2$) to water slurry of casein curd in wet or dry form and subsequently dried. The final pH of the caseinate solution should not exceed pH 6.8.

Caseinates possess good functional properties, such as solubility in water and milk, stabilizing effect being important in yoghurt manufacture, emulsification capacity and foamability. The taste of caseinates must be neutral. The amount of added caseinates to yoghurt milk should not exceed 2.0 per cent, because higher doses may result in undesirable thickening of yoghurt (Gennip, 1974).

2.4.3.3 Preparation of Soluble Coprecipitates

Coprecipitates are produced by the simultaneous coagulation of casein and whey protein in skim milk. The curd is washed, dried and ground. The properties of isoelectric coprecipitates resemble those of casein since its composition is about 80 per cent of the casein protein.

A solubilized coprecipitate is manufactured by treating a slurry of isoelectric coprecipitate with an alkali and spray drying of this solution. The final powder has characteristics similar to those of sodium caseinate.

2.4.3.4 Whey Protein Preparation

The soluble whey protein preparations are produced mainly by ultrafiltration of the whey resulting in the high quality product.

Rham (1984) described the process for the production of whey protein preparations which are heat-stable at neutral pH and are suitable for increasing the protein content of beverages or infant formulas.

Moreno and Lycak (1974) suggested a method for producing heat- and acid-stable whey protein, suitable as an additive to acid beverages with a pH of 4.0 or below. In a typical example, the whey protein is prepared by:

i) Adjusting the pH of liquid whey to 7.0 by adding conc. NaOH solution;

ii) Heating the whey to 65°C for 15 min., centrifuging it and cooling it to room temperature;

iii) Recovering the supernatant and adjusting the pH to 6.5 by the addition of phosphoric acid;

iv) Freeze drying the liquid to produce a solid material containing (on a dry basis) 12.1 per cent protein, 66.0 per cent lactose and 12.3 per cent ash;

v) Reconstituting the freeze-dried material to a total solids content of 25 per cent;

vi) Passing it through a Sephadex G-25 column; and

vii) Freeze-drying the effluent to produce a product containing 67.3 per cent protein, 13.2 per cent lactose, and 6.3 per cent ash, in addition to 0.77 per cent Ca and 1.76 per cent P.

The freeze dried product had been incorporated successfully in grape or orange-flavoured beverages of pH 4.0 or below, which were subsequently heat-treated and carbonated without any adverse effects.

2.4.4 PREPARATION OF SOLUBLE DISPERSION OF PROTEIN

Yasumatsu et al. (1980) developed a method for the production of milk containing acid syrup which had a stable dispersion of protein and is consumed by diluting with water or carbonated water for drinking. The milk containing acid syrup was produced by adding sugar (20-52% w/w) to an acidified milk which satisfied the following conditions.

$$y \leq -0.27_x + 4.15$$

where, x denotes the casein content in the acidified milk and y, the pH value of the acidified milk.

2.4.5 PROCESSING FACTORS AND PROTEIN STABILITY

Hooydonk et al. (1982a) investigated the influence of processing factors with reference to the viscosity and stability of drinking yoghurt at 20^{o}C. They concluded that apart from the use of a final pasteurization temperature of 100^{o}C and considerably reduced viscosity and stability, none of these factors had any major effects. Single, pasteurization at 15 MPa was sufficient when the yoghurt and pectin had been mixed carefully.

Kim et al. (1984) studied the method and level of addition of acid for development of sour milk. To make acid milk gelled beverages, sugar, carboxymethyl cellulose and gelatin were added at the rate of 9.0, 0.6

and 0.5 per cent, respectively to whole milk, skim milk or diluted milk (milk/water 2:1), and citric or lactic acid were added. When the acids were added rapidly, protein was precipitated in all samples (when acid concentration was more than 0.5 per cent). When they were added slowly, some samples were free of precipitation even 1.0 per cent acid was added. In skim milk and whole-milk, the gel increased as acid concentration increased, but in case of diluted milk gel was not formed. Products made from whole milk and with 0.5 per cent acid (0.25% lactic plus 0.25% citric acid) received the best scores.

2.5 APPLICATION OF HYDROCOLLOIDS IN LOW-pH MILK-BEVERAGES

Stabilizers are generally polysaccharides in nature and function through their ability to form gel structure in water or due to their ability to combine with water as water of hydration. Most of the stabilizers and thickeners used in the food industry are derived from natural sources although some are chemically modified to achieve specific characteristics. Since all effective stabilizers are hydrophilic, and are dispersed in solutions as colloids, they are usually referred to as hydrocolloids.

The general properties of these hydrocolloids include significant solubility in water and a capability to increase viscosity. The efficiency of these materials in many food applications is directly dependent on their ability to increase viscosity, to stabilize suspensions and improve textures. Hydrocolloids do not, however, function as emulsifiers since they lack the necessary combination of strong hydrophilic and lipophilic properties.

Stabilizers are of importance in a number of dairy products like flavoured milk beverage, fruit butter milk drinks, fruit skim milk yoghurt, fruit milk drinks and fruit mix drinks based on sweet or soured whey (Anon, 1976).

The market development of extended shelf-life dairy products generally, at low pH varieties, has resulted specifically in the need for stabilizer systems. It is well known that at low pH values (less than 4.6, the isoelectric point of casein), it is not practical to heat-treat dairy products without denaturation of the protein fractions and their subsequent flocculation, separation and precipitation. Selected hydrocolloids like high methoxylated pectins, carboxymethyl cellulose and propylene glycol alginate, are able to function as a protective colloid in low-pH milk-products (Gregory, 1985).

2.5.1 ROLE OF STABILIZERS ON THE SENSORY PROPERTIES OF LOW-pH MILK BEVERAGE

The sensory properties of any food are also of utmost importance like their chemical and microbiological properties as well as nutritive value. The sensory properties are dependent not only on the composition, quality of raw materials and processing parameters but also on several other factors, such as body and texture, flavour and colour & appearance, mouthfeel etc. Many emulsifiers and stabilizers enhance the sensory properties of food products. They mostly improve the body and texture of the finished products, but their effect on flavour is indirect.

The properly designed stabiliser system for the products made by UHT processing would allow protein stability, emulsion stability, suspending capability, syneresis prevention, prevention of 'wheying off', gelling ability, and mouthfeel and bodying properties (Glicksman, 1983).

2.5.1.1 Pectins

High methoxylated pectins can effectively stabilize soured milk products (Nelson, 1979). The protein stability of acid milk beverages containing natural grape juice (Hah, 1975), fruit-mash (Arolski et al., 1977), cheese-whey with orange emulsion and flavour (D'Yachenko and Sqares, 1984) and whey with fruit juice (Iverson, 1984) were improved successfully by adding pectins singly or in combination with other stabilizers.

2.5.1.3 Starch

Starches especially the modified ones, have also found wide application in ultra high treated dairy products as thickening and gelling agents. Modified starches have been developed that will maintain low viscosity during processing and packaging but will then set up in the container to give a good gelled pudding texture (Anon, 1981b). The starch is also reported to be used for improving mouthfeel but actually it is being used as cheap filler for the cocoa based drinks (Morley, 1983; Werry, 1984).

2.5.1.4 Carboxymethyl cellulose (CMC)

Carboxymethyl cellulose is produced by chemical modification of cellulose and is available in several forms. It has been used in conjenction with carrageenan or propylene glycol alginates to stabilize milk drinks of pH 4 to 4.5 and to impart a rich body and mouthfeel (Werry, 1984). Shenkenberg et al. (1971) developed the formula for stabilized milk-orange juice beverage, containing 0.2 per cent CMC.

Carboxymethyl cellulose (sodium salt) can also be used in the fruit flavoured milk beverage (Nishiyama, 1976; Nishiyama, 1982). CMC in combination with gelatin (Igoe, 1976), with xanthan gum (Daehler, 1979) and with pectin ('D'Yachenko and Sqares, 1984) were found to be helpful in the preparation of acidified milk beverages.

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2.5.1.5 Other Gums

Locust bean gum is derived from the carob bean kernel. Although locust bean gum lacks gelling properties, its viscosity is little affected at a wide range of pH and is usually used in conjunction with carrageenan for low pH milk drink (Ilany, 1977). A stable (without curd separation) acidified whole milk beverage (with a pH of 3.4-3.6) was prepared using 0.3 per cent locust bean gum (Takahata, 1980; Anon, 1980a; Anon, 1981c).

Guar gum (Ilany, 1977) can be obtained from seeds of the guar plant and has properties and uses similar to those of locust bean gum. The main difference is that guar gum will hydrate in cold water more completely and more rapidly than locust bean gum.

Xanthan gum is produced by microbial action on dextrose and being usually stable to prolonged heating at low-pH, has been used in low pH milk drink stabilization (Warry, 1984). Daehler (1979) developed new milk based drink (pH 4.1-4.5) which contains 0.1 per cent xanthan gum, 0.14 per cent carrageenan and 0.40 per cent carboxymethyl cellulose.

2.6 APPLICATION OF STABILIZING AND EMULSIFYING SALTS IN LOW-pH MILK BEVERAGE

In the manufacture of all emulsion based products, the main concern is to stabilize the emulsion, so that it does not break down or separate upon standing for a reasonable period of time. Emulsifiers play an important role in the process of emulsification and in preparing stable food emulsions.

Typical emulsifiers used in dairy industry are: lecithin, mono- and di-glycerides, fatty acids esters of polyhydric alcohols and different salts such as disodium phosphate, trisodium citrate and sodium tartrate. Both stabilizers and emulsifiers play an important role in enhancing the body and texture of the dairy products. They also help to improve the flavour indirectly and have negligible influence on food value.

Addition of tri-sodium citrate (0.03%) in flavoured beverages, before heating is reported to impart very good cloud, and frothing capacity with a very creamy taste (Hawthorne et al., 1977).

Schanderl and Hedrick (1968) prepared stable cherry-milk beverage, having a shelf life of at least two weeks. The system was stabilized with the addition of dibasic potassium phosphate and pasteurized at 145^{o}F for 30 min. The consumer acceptance of both stabilized or unstabilized cherry-milk was good, as measured by panels and initial store sales trials.

To reduce surface tension and allow the emulsion to be formed more easily, hydrocolloids like gum arabic are employed. Gum arabic is known as very effective emulsifying agent and used widely for making flavour emulsions for beverage and bakery applications (Glicksman, 1983).

2.7 CONCLUSIVE REMARKS

It is evident from the information collected on composition and manufacturing techniques on various types of acidified milk based beverages, considerable attention had been paid to improve the stability of protein system by selection of suitable stabilizers. Further it is noticed that different types of ingredients and acidulants are used in the preparation of acidified beverage. The effect of using different ingredients have definite effect on the organoleptic quality of the product as well. It is therefore, necessary to study in depth about the effect of using different types of ingredients and processing parameters in the preparation of acidified milk based beverages to assess the final quality, especially the organoleptic quality.

CHAPTER - 3

SCOPE AND PLAN OF WORK

3. SCOPE AND PLAN OF WORK

3.1 SCOPE OF STUDY

The foregoing chapter on review of literature would reveal that the published information on the various aspects of production and physico-chemical properties of directly acidified milk beverage is scanty. Many of the lactic beverages reported have whey as the basic raw material and only few reports appear on use of milk, skim milk or butter milk. The acidic beverage is essentially prepared by using specific cultures. Information on directly acidified milk beverages, if any, is mostly given only in the patent forms. In the preparation of milk based acidified beverages, stabilization of protein poses problem. However, the existing knowledge on stabilizing the protein system using different types of stabilizers can be advantageously utilized in developing the milk based directly acidified soft drinks and beverages. Further adjustment of solids level in the beverage is necessary to develop a product of acceptable quality. It is also reported that mouthfeel of the beverages could be improved by the use of proper combinations of different gums and stabilizers. Therefore, it is necessary to make investigation on use of approved stabilizers in improving the sensory characteristics of acidified beverages.

The shelf life of any food product is enhanced considerably by suitable heat treatment and with the use of suitable packaging materials. Presently many flexible metallised laminated materials are available in the market for packaging of different products. It would be worthwhile therefore to compare the performance of any typical material as against the use of conventional glass bottles for sterilized beverages.

Technology also needs to be standardized for enhancing the shelf life of acidified milk beverage by selecting appropriate sterilization time-temperature combination.

It is hoped that this information may be useful for dairy industry in the manufacture of such a new dairy beverage with an acceptable quality and longer shelf-life.

The present study was, therefore initiated for the production of acidified milk beverage with the following objectives:

1. Standardization of the manufacturing technique of the acidified milk beverage;

2. To study the physico-chemical properties and microbiological quality of beverage.

3. To study the shelf-life of the beverage at $30^{o}C$ and $5^{o}C$ stored in suitable packaging materials; and

4. To compute the cost of production of the developed beverage.

3.2 PLAN OF WORK

To achieve the above stated objectives, the plan of work was drawn as follows.

3.2.1 DEVELOPMENT OF MANUFACTURING PROCESS

3.2.1.1 Selection of pH-sugar Combination and Type of Acid

Laboratory trials were conducted to evaluate suitable level of pH and sugar combinations based on flavour score of product. For this purpose, following pH and sugar levels were tried in the acidified beverage using four types of acid.

a) pH level : 4.50, 4.25, 4.00, 3.75 and 3.50

b) Sugar level : 14, 16, 18 and 20 gm per 100 ml of standardized (i.e. toned or diluted) milk.

c) Type of acid : Citric, phosphoric, lactic and hydrochloric acid.

Based on the flavour scores, better acceptable combination for each type of acids were selected for further optimisation of pH and sugar levels in the beverage.

3.2.1.2 Computation of Time-temperature Combination of Sterilization

The suitable time-temperature combinations for selected pH-sugar level combinations were computed by interpolating the data given by Srimani and Loncin (1980) for effective sterilization of the product.

3.2.1.3 Selection of Stabilizer

Trials were conducted to find the suitable levels and type of stabilizer based on their effect on visual observation on protein stability, viscosity and sensory score. For this purpose, following stabilizers of different grades were tried.

a) Pectin (Sigma and local)

b) CMC-Na salt (Sigma-low viscosity, Sigma-medium viscosity and local)

c) Guar gum (local MV 2/4 grade and MV 2/3 grade)

d) Locust bean gum (Sigma)

e) k-Carrageenan (Sigma)

f) Agar (Sigma)

g) Pregelatinized potato starch (local)

Final selection of stabilizer was done on the basis of development of minimum viscosity and maximum stability of protein system at the sterilization conditions of the product.

3.2.1.4 Optimisation of Level of Milk Solids

For getting a product with acceptable consistency, the toned milk (3% fat and 8.5% SNF) was diluted with water (Toned milk : water) to the following ratio.

80:20, 70:30 and 60:40

The selected level of stabilizer was added to the diluted milk in a proportionate quantity on the basis of milk dilution.

The beverage samples prepared with diluted milk were evaluated on the basis of body and texture, and flavour scores. The viscosity of the samples were also measured.

3.2.1.5 Selection of Stabilizing Salt

Following salts were used in diluted toned (70:30) milk at the rate of 0.06, 0.09 and 0.12 gm per 100 ml of diluted milk.

a) Tri-sodium citrate.

b) Di-basic potassium phosphate.

Selection of suitable level of salt was done on the basis of flavour, and body and texture scores.

3.2.1.6 Selection of Added Flavour

Following flavours were used at three different levels viz. 0.5, 1.0 and 1.5 ml/litre of beverage.

a) Pineapple.

b) Lemon.

c) Strawberry.

Most suitable type and level of added flavour was selected on the basis of flavour scores only.

3.2.1.7 Type of Added Colour

Lemon yellow colour was used, and their desired intensity in the product was evaluated on the basis of consumer acceptance trial.

3.2.2 STORAGE STUDY

3.2.2.1 Packaging Materials

a) Glass bottles.

b) Metallised polyester pouches.

3.2.2.2 Storage Temperatures

a) $30^{o} \pm 1^{o}C$ (Controlled temperature)

b) $5^{o} \pm 2^{o}C$ (Refrigeration temperature)

3.2.3 PRODUCT QUALITY EVALUATION

3.2.3.1 Sensory Attributes

a) Flavour.

b) Body and Texture.

c) Colour and appearance.

d) Overall acceptability.

3.2.3.2 Physico-chemical Parameters

a) pH.

b) Viscosity.

c) Sediment.

d) Colour (reflectance).

e) Total solids.

f) Fat.

g) Protein.

h) Free fatty acids.

i) Thiobarbituric acid value.

j) Non-protein nitrogen.

k) Reducing sugar.

l) Total HMF value.

3.2.3.3 Microbiological Attribute

a) Total spore count.

3.2.4 CONSUMERS ACCEPTABILITY OF ACIDIFIED MILK BEVERAGE

3.2.5 COST OF PRODUCTION

The cost of production of standardized acidified milk beverage was estimated.

MATERIALS AND METHODS

This chapter deals with the materials and methods used in standardizing the process for the manufacture of acidified milk beverage and also the methods used for the analysis of milk and acidified milk beverage. Only analytical grade reagents were used unless mentioned otherwise.

4.1 MATERIALS

4.1.1 INGREDIENTS

4.1.1.1 Milk

The fresh, pooled raw buffalo milk obtained from the Experimental Dairy of the Institute was used for the present study.

4.1.1.2 Sugar

Commercial grade white crystalline sugar drawn from stores, Experimental Dairy, National Dairy Research Institute, Karnal was used in all trials.

4.1.1.3 Stabilizers

The following stabilizers were used in the present studies.

a) Pectin-(Sigma) (from Citrus fruit, Sigma Chemicals Co., USA).

b) Pectin-(Local) (Rapid Set 150^{o}C) Jaydip Agencies, Bombay.

c) Carboxymethyl cellulose (CMC) (Sodium salt - low viscosity) Sigma Chemical Co., USA.

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d) Carboxymethyl cellulose (Sodium salt - medium viscosity) Sigma Chemical Co., USA.

e) Carboxymethyl cellulose (Sodium salt - medium to high viscosity) Loba Chemical Co.

f) Guar gum (MV - 2/4 grade) Hindustan Gums Ltd., Bhiwani.

g) Guar gum (MV 2/3 grade) Hindustan Gums Ltd., Bhiwani.

h) Locust bean gum (Sigma Chemical Co., USA).

i) k-Carrageenan (Sigma Chemical Co., USA).

j) Agar (Sigma Chemical Co., USA).

k) Pregelatinized potato starch (Chemical de Centre, New Delhi).

4.1.1.4 Stabilizing Salts

a) Tri-sodium citrate (E.Merck (India) Pvt. Ltd., Bombay).

b) Di-basic potassium phosphate (BDH Lab. Chemical)

4.1.1.5 Acids

a) Citric acid (Food grade citric acid supplied by Citurgia Biochemical Ltd., Ballard Estate, Bombay).

b) Phosphoric acid (Food grade ortho-phosphoric acid supplied by Jaydip Agencies, Bombay).

c) Lactic acid (Sarabhai M. Chemicals, Gorwa Road, Baroda).

d) Hydrochloric acid (Sarabhai M. Chemicals, Gorwa Road, Baroda).

4.1.1.6 Flavours

In the present study, the following edible grade flavours manufactured by M/s. Bush Boake Allen (India) Ltd., Madras, were used.

a) Soluble essence pineapple No.1

b) Soluble essence Lemon No.1

c) Soluble essence strawberry No.1

4.1.1.7 Colour

The following edible grade colour manufactured by M/s. Bush Boake Allen (India) Ltd., Madras, was used.

a) Lemon yellow powder, IH 6597.

4.1.2 EQUIPMENTS

4.1.2.1 Heating/Pasteurization Vat

A 20 litre capacity, stainless steel jacketed, round bottom, open pan provided with a steam line and steam control valve was used for the preparation of beverage samples.

4.1.2.2 Homogenizer

'Rannie' single stage homogenizer was used for homogenization of the acidified milk beverage during initial phases of the studies for laboratory trials for selection of suitable level and combination of acid, pH, sugar and stabilizer. In later part of the studies, 'Crepaco' two-stage homogenizer was used.

4.1.2.3 Autoclave

A laboratory autoclave (manufactured by Medica Instrument Mfg. Co., Bombay) was used for sterilization of the acidified milk beverage.

4.1.3 PACKAGING MATERIALS

4.1.3.1 Glass Bottles

Sterilizable glass bottles (200 ml capacity) were purchased from local market.

4.1.3.2 Metallised Polyester Pouches

This three ply metallised polyester laminate consisted of polyester-film (50 gauge), aluminium foil (0.0254 mm) and low density polyethylene film (150 gauge). Pouches were supplied by M/s. Flex Laminates, Ghaziabad, U.P.)

4.2 METHOD OF MANUFACTURE

Attempts were made to standardize the method for the manufacture of acidified milk beverage. Different parameters taken for study for effecting process. With this procedure several modification and standardization included different pH and sugar levels, types of acid, stabilizers and emulsifiers level, milk solids (i.e. fat and SNF level), different types of flavour and their compatible colour.

4.2.1 STANDARDIZATION OF MILK

Fresh, pooled raw buffalo milk obtained from

Experimental Dairy of National Dairy Research Institute, Karnal was filtered through muslin cloth and about half of the quantity was separated for obtaining skim milk. Remaining portion of milk was then standardized to desired level of fat and SNF with skim milk and water for different experimental conditions.

4.2.2 MIXING THE INGREDIENTS

Calculated amount of stabilizers and emulsifiers were mixed with portion of sugar separately. Standardized milk was warmed upto 45°C, then sugar added to milk gradually at a temperature of 45°-50°C with constant stirring. The mixture was further heated to 70°-75°C. This was followed by the addition of sugar-stabilizer-emulsifier blend with constant stirring.

4.2.3 COOLING AND ACIDIFICATION

The milk mixture was initially cooled by dipping the can in tap water to about 30°C. They were further cooled to about 6°-8°C by dipping the can in chilled water tank. The pH of the mixture was then adjusted to the required level by adding acid solution (slowly) with continuous stirring. The acidified milk mixture was then kept at low temperature for about 30 minutes. In case of initial laboratory trials for optimisation of pH levels without addition of stabilizer to the milk, it was chilled (6°-8°C) to minimize the intensity of coagulation.

4.2.4 PREHEATING AND HOMOGENIZATION

In order to avoid the fat separation in the product and to get a uniform body and texture, mixture was homogenized resulting in breaking of fat globules. All the samples were heated to 60°C and homogenized either in 'Rannie' single stage homogenizer or in 'Crepaco' two stage homogenizer at pressure of 2500 and 500 psi.

4.2.5 ADDITION OF COLOUR AND FLAVOUR

Required quantity of colour solution was added to the mixture just before homogenization and required amount of flavour was added before packaging of the product.

4.2.6 PACKAGING AND STERILIZATION

After homogenization, the product was filled into 200 ml clean glass bottles and in metallised polyester pouches (200 ml). Glass bottles were sealed with crown cap whereas pouches were heat-sealed using hand operated impulse sealer. Sealed bottles and pouches were then sterilized in laboratory autoclave at required time and temperature.

4.2.7 COOLING

Bottles and/or pouches were removed carefully from autoclave and cooled gradually at room temperature.

4.3 PROCESS OPTIMISATION

In order to optimise the process for the manufacture of acidified milk beverage, the following parameters were studied.

4.3.1 SELECTION OF pH-SUGAR COMBINATION AND TYPE OF ACID

Laboratory trials were conducted to determine suitable pH and sugar level combinations on the basis of sensory score in acidified milk beverage.

For this purpose, beverage samples were prepared using three types of acid at different pH and sugar level combinations as mentioned in Table 4.1. On the basis of extensive trials conducted in the

Table 4.1: Type of acid, their concentration, and pH and sugar level used during investigation.

Type of acid	Approximate concentration	pH	Sugar level (% w/v of toned or diluted milk)
	10.50 (% w/v)	4.25	14, 16, 18 and 20
Citric	13.60 (% w/v)	4.00	14, 16, 18 and 20
	18.20 (% w/v)	3.75	14, 16, 18 and 20
	1.825 N	4.00	14, 16, 18 and 20
Phosphoric	2.250 N	3.75	14, 16, 18 and 20
	2.400 N	3.50	14, 16, 18 and 20
	9.50 (% v/v)	4.50	14, 16, 18 and 20
Lactic	11.90 (% v/v)	4.25	14, 16, 18 and 20
	12.85 (% v/v)	4.00	14, 16, 18 and 20

Note: These levels of different acids were selected on the basis of preliminary laboratory trials.

laboratory, strength of different acid solutions to be used in the investigation were standardized. Standardized solutions were then slowly added to milk mixture at the rate of 8.0 ml/100 ml of standardized milk during throughout investigation.

4.3.2 COMPUTATION OF TIME-TEMPERATURE COMBINATION OF STERILIZATION

Sterilization by heat is a well known process. The time-temperature combination used for conventional sterilization of milk is usually 121°C for 10-15 min. The selection of time-temperature is based on the thermal death rate values (D values) for a given reference organism e.g. Bacillus stearothermophilus. The D values are dependent on the temperature of sterilization and pH of sterilization medium. Thus, the sterilization time at a given sterilization temperature can be predicted from the relationships of D value with temperature and pH.

The thermal death rate values for acidified milk beverages were obtained from the following D value-pH relationship given by Srimani and Loncin (1980).

$$\text{Log } D = 0.533844 \text{ pH} + 0.550688$$

The D values of 660, 485.4 and 356.9 sec. at 100°C were obtained for acidified milk beverages of pH 4.25, 4.06 and 3.75 respectively. Using these D values, the time required for sterilization at 100°C (for 9 logcycle reduction of <u>B.stearothermophilus</u> spore) was worked out, which was found to be 99, 72.8 and 53.5 minutes for acidified beverages of pH 4.25, 4.00 and 3.75, respectively. The time required for the same sterilization effect at 110°C was worked out using Q_{10} value of 11.0 (Z = 9.60°C) (Miller and Kandler, 1967) and the following relationship.

$$tn = \frac{t_i}{Q_{10}^{\,n-1}}$$

where,

tn = time in minutes at 110°C

t_i = time in minutes at 100°C

n = number increment in temperature in 10°C.

Thus, the sterilization time required at 110°C for acidified beverages of pH 4.25, 4.00 and 3.75 worked out to be 9.00, 6.62 and 4.86 min. respectively. These values were rounded off to 9, 7 and 5 minutes.

It was expected that heat treatment of 110°C for 9, 7 and 5 min. would be sufficient to obtain a sterilized product with the pH values at 4.25, 4.00 and 3.75 respectively. Since the product prepared with using phosphoric acid at pH 3.75, was found acceptable to the judges, it was considered for further investigation and hence sterilization time-temperature combination (i.e. 110°C for 5 min) was tested further to confirm efficacy of sterilization effect. For this purpose, samples with pH 3.75 were prepared and heat treatment of 110°C for

5 min was given. The samples were then tested for total spore count as per the method recommended in IS: 4238 (1967). In the results, it was observed that none of the beverage samples in 10 trials were found to have even a single spore and hence this time-temperature combination was considered for acidified milk beverage during further investigation.

4.3.3 SELECTION OF STABILIZER

Laboratory trials were conducted for selecting suitable type or level of stabilizers on the basis of visual body and texture characteristics, and protein stability during sterilization process. For this purpose different types of stabilizers at different levels were tried in milk samples. The levels, and types of stabilizers used in the present study were as per the list given below.

a) Pectin (Sigma) 0.2, 0.3, 0.4 and 0.8%.
b) Pectin (local) 0.2, 0.3, 0.4 and 0.8%
c) CMC - low viscosity (Sigma) 0.2, 0.3, 0.4 and 0.8%.
d) CMC - medium viscosity (Sigma) 0.2, 0.3, 0.4 and 0.8%.
e) CMC (local) 0.2, 0.3, 0.4 and 0.8%.
f) Guar gum - MV 2/4 grade (local) 0.3%.
g) Guar gum - MV 2/3 grade (local) 0.3%.
h) Locust bean gum (sigma) 0.3%.

i) k-Carrageenan (Sigma) 0.03%.

j) Agar (Sigma) 0.3%.

k) Pregelatinized potato starch 0.3%.

In all trials, ~~standardized~~ toned milk (3.0% fat and 8.5% SNF) was used. Individual lot of milk was mixed with sugar at the rate of 10 gm/100 ml of standardized milk and calculated amount of stabilizers were dissolved. The mixture was then sterilized in an autoclave.

The most suitable type of stabilizer was selected on the basis of visual observation of protein stability and viscosity of the product.

4.3.4 OPTIMISATION OF LEVEL OF MILK SOLIDS

Toned milk (3% fat, 8.5% SNF) when used for preparation of acidified milk beverage, it was found that the viscosity of the final product was very high, and hence may not be acceptable as a beverage. It was therefore, felt necessary to reduce the viscosity of the product by using diluted milk. For this purpose, three levels of dilution viz. 80:20, 70:30 and 60:40 with water were used. The addition of stabilizer for diluted toned milk was done in proportion to milk solids and samples of acidified milk beverage were prepared. Selection of milk dilution required was done on the basis of sensory evaluation (i.e. flavour, and body and texture score) and viscosity measurement of the beverage samples.

4.3.5 SELECTION OF STABILIZING SALT, FLAVOUR AND COLOUR

Once the process was standardized for the preparation of sterilized and stable acidified milk beverage with suitable combination of pH and sugar level, stabilizer and milk solids level, it was further improved by the addition of stabilizing salt, flavour and acceptable colour at different levels (section 3.2.1.5, 3.2.1.6 and 3.2.1.7, respectively).

4.4 PACKAGING AND STORAGE

4.4.1 PACKAGING

In order to study the stability of beverage under different storage conditions, it was packed in glass bottles (capacity, 200 ml) and metallised polyester pouches (200 ml capacity). Glass bottles were closed tightly with crown cap with the help of capping machine while metallised polyester pouches were heat sealed. Bi-axially orientated polypropylene (BOPP) films were tried initially, but was not found suitable for packaging acidified milk beverage, hence these were dropped from experimental study.

4.4.2 STORAGE

The packaged samples were then stored at $5^{o} \pm 2^{o}C$ (90 ± 5% RH) in a refrigerated cabinet and in a separate thermostatically controlled incubator maintained at $30^{o} \pm 1^{o}C$ (70 ± 5% RH).

The stored product was analysed at regular intervals for various physico-chemical properties viz. pH, sediment, viscosity, colour (reflectance), reducing sugar, free fatty acids, TBA value, non-protein nitrogen and HMF value. It was also subjected to sensory evaluation and total spore count.

4.5 ANALYTICAL METHODS

4.5.1 ANALYTICAL METHODS FOR MILK AND SKIM MILK

4.5.1.1 Fat

Fat content of milk and skim milk were determined by Gerber method as described in IS:1224, Part I - First revision (1977).

4.5.1.2 Milk Solids-not-fat (SNF)

The SNF content in milk and skim milk were determined by zeal lactometer using Richmond's formula as under.

$$SNF\ (\%) = \frac{CLR}{4} + 0.2\,F + 0.14$$

where,

'CLR is the corrected lactometer reading at 29^{o}C, and 'F' is the per cent fat in milk.

4.5.2 ANALYTICAL METHODS FOR ACIDIFIED MILK BEVERAGE

4.5.2.1 Fat

Fat percentage in acidified milk beverage was estimated by Rose-Gottlieb method (IS: 2802, 1964) using Mojonnier fat extraction apparatus (IS: 2311, 1973).

4.5.2.2 Total solids

The total solids content of beverage was determined by the Indian Standard method (IS: 2802, 1964). About 5.0 gm of beverage sample was weighed accurately in a clean-dried metal dish containing about 20 gm of prepared sand and a stirring rod. The sand was further saturated by careful addition of a few drops of double distilled water, and thoroughly mixed. The wet sand with sample was then spread uniformly in the drying dish. The dish containing the sample was then kept in an electric oven maintained at $102^{o} \pm 1^{o}C$ until a constant weight of dried dish is obtained. Total solids content was calculated in the per cent by weight, as per the formula given below.

$$\text{Total solids (\%)} = \frac{100 \times (C - A)}{(B - A)}$$

where,

A = weight of empty dish (in gm) along with sand and glass rod;

B = weight of dish (in gm) with the sample before drying, and

C = weight of dish (in gm) with the sample after drying.

4.5.2.3 pH

pH of samples, during product development and storage study was determined by the digital pH meter (Model, MDH-5, Mercantile Engineers, New Delhi) at 20°C temperature.

4.5.2.4 Total Protein

The total nitrogen content of the sample was determined by the method of Meneffee and Overmann (1940), with slight modifications, wherein an accurately weighed (about 0.5 gm) sample was transferred into a 300 ml Kjeldahl flask and digested after the addition of 2.0 gm sodium sulphate, 0.14 gm mercuric oxide and 5 ml conc. sulphuric acid, for 45 min. or till the mixture became clear. The content of the flask were cooled, 50 ml nitrogen free distilled water added to it and the ammonia distilled off, with 15 ml of 50 per cent (w/v) sodium hydroxide solution, into 25 ml saturated boric acid solution containing 4 drops of mixed indicator (made by dissolving 100 mg methyl red and 30 mg methylene blue in 60 ml of 95 per cent ethyl alcohol and then making up the volume to 100 ml with distilled water). Approximately 60 to 70 ml distillate was collected in a 100 ml conical flask. The contents of the flask were titrated against 0.02 N sulphuric acid. A blank determination, using distilled water in place of sample was also carried out. The total nitrogen and per cent protein were calculated as under.

$$\text{Per cent nitrogen} = \frac{(A - B)\ N \times 14.0 \times 100}{W}$$

where,

A = ml sulphuric acid required for sample

B = ml sulphuric acid required for blank

N = normality of sulphuric acid used

W = weight of sample in mg

Per cent protein = per cent total nitrogen x 6.38

4.5.2.5 Viscosity

The dynamic viscosity of acidified milk beverage was determined by using Hoeppler's falling ball viscosimeter (manufactured by Veb Prufgeratewerk Medingen/ Dresden, Germany). All the samples were tempered and kept constant at 20°C. The desired temperature was maintained by circulating water through the outer jacket of the viscosimeter. Hoeppler viscosimeter was levelled by using the spirit level. The measuring tube was filled with the sample and the ball was inserted through the open end of the tube. Care was taken to avoid air bubbles in the tube and the open end was closed. Different balls were used for various samples depending on the requirement. The time taken by the ball to fall through a distance to 100 mm was measured accurately in consecutive runs by means of a 1/10 second stop-watch and the mean was calculated.

The specific gravity of sample was also determined at 20°C using a specific gravity bottle.

The dynamic viscosity of the sample was calculated by the formula based on the Stoke's law.

$$\eta = F (S_K - S_f) K$$

where,

η = dynamic viscosity in centipoise

F = fall time of the ball in seconds (angle of inclination, 80^0)

S_K = specific gravity of the ball

S_f = specific gravity of the sample, and

K = ball constant

4.5.2.6 Colour (Reflectance)

The colour of the product was measured in terms of reflectance using a "Reflectometer CL-28" (*Elico Pvt. Ltd., Hyderabad, India*).

The operating procedure consisted of setting indicator at 'zero' by adjusting screw provided in the meter. Instrument was then connected with main supply (220/240 V) and switch was set to 'on' position. With blank plate in the socket, indicator was readjusted to 'zero' with the 'set-zero' knob. Search unit was then placed on the white standard magnesium oxide block and instrument was adjusted to the value corresponding to the reflectivity marked on the block with the aid of the sensitivity knob. Magnesium oxide block was then replaced with black, grey and white slabs supplied with the instrument and adjustment made to ensure that the meter reading corresponds to the reflectivity values marked on these slabs. When everything was in order, beverage sample in glass bottle (rectangular) was placed before the search unit and reflectivity measured at 450 nm and the scale reading noted. Per cent change in reflectance was obtained by using following formula.

$$\text{Per cent change in reflectance} = \left\{\frac{X.100}{Y}\right\} - 100$$

where,

X = reflectance of stored sample, and

Y = reflectance of fresh sample

4.5.2.7 Sediment

The sediment content was determined by the Indian Standard Method (IS: 1479, Part II, 1961) with some modification. Ten ml of sample (at 24°C) was taken in 15 ml capacity graduated centrifuge tube (0.1 ml graduation) and centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded and replaced with equivalent amount of distilled water and centrifuged again for 10 minutes at 2000 rpm. The amount of solids, in ml, remaining in the centrifuge tube was taken as sediment.

4.5.2.8 Reducing Sugar

The procedure prescribed for ice-cream by Indian Standard Institution (IS: 2802, 1964) was followed to determine reducing sugar in acidified milk beverage wherein about 10 gm of sample was taken.

4.5.2.9 Free Fatty Acids (FFA)

The method of Deeth *et al.* (1975) was adopted for estimation of free-fatty acids with slight modification.

Ten ml of well prepared beverage sample at room temperature was transferred into a 35 ml stoppered test tube and to this 10 ml of extraction mixture (iso-propanol:petroleum ether: 4N H_2SO_4 in 40:10:1 proportion) was added and mixed well by vigorous shaking. Petroleum ether (12 ml) and water (8 ml) were added and stoppered the test-tube and shaken vigorously for 15 seconds. The two layers were allowed to settle (within 5-10 minute) and an aliquot of the upper layer was withdrawn carefully and transferred to a 50 ml conical flask. After addition of 6 drops of 1 per cent methanolic phenolphthalein indicator, the solution was titrated with 0.02N methanolic KOH. A blank in which product was replaced with water, was used to obtain the background titration. The free fatty acids content of beverage sample was calculated in terms of micro equivalent per millilitre (μ eq/ml) of beverage, as follows.

$$FFA\ (\mu\ eq/ml) = \frac{T \times N}{P \times V} \times 10^3$$

where,

T = net titration volume (in ml) of methanolic KOH

N = normality of methanolic KOH (0.02 N)

V = volume of beverage sample (in ml), and

P = proportion of the upper layer titrated

$$\text{i.e.} \quad \frac{\text{Volume of aliquot withdrawn for titration}}{\text{Total volume of upper layer}}$$

4.5.2.10 Thiobarbituric Acid (TBA) Value

The TBA value was estimated as per the method described by Sidwell et al. (1955) with slight modifications. Ninety ml of well mixed acidified milk beverage sample was transferred into 800 ml of Kjeldahl flask and to this 4.2 ml of 3 N HCl was added. This was thereafter distilled and 50 ml of distillate was collected in 10 min. From this, 10 ml distillate was transferred into 25 ml glass-stoppered tube. After adding 1.0 ml TBA reagent (0.67 gm/100 ml glacial acetic acid), the tube was placed in boiling water bath exactly for 35 min., cooled to room temperature and the absorbance (OD) was measured at 530 nm against distilled water as a blank in a spectrophotometer (Spectronic 20, Bausch and Lomb, USA).

4.5.2.11 Non-protein Nitrogen (NPN)

The NPN content of acidified milk beverage was determined by the method of Rowland (1938) with slight modification. Ten ml of sample was taken into 100 ml volumetric flask. Diluted with 25 ml of nitrogen free water. 2.5 ml of lead acetate (41%) solution was then added to precipitate the sample. Water was then added upto the mark and mixed thoroughly. When precipitate settled at the bottom, supernatant liquid was filtered through Whatman No.42 filter paper and 20 ml of clear filtrate was used for nitrogen estimation as per 4.5.2.4).

4.5.2.12 Total 5-Hydroxy Methyl Furfural (HMF) Value

The method suggested by Kenney and Bassette (1959) was employed for the determination of HMF value with some modification.

Ten ml of beverage sample (without added colour) was taken in 50 ml test tube, followed by addition of 5 ml of 0.3 N oxalic acid. Tube was covered and placed in a boiling water bath for one hour. After this, it was removed and cooled to room temperature with tap water. This was followed by the addition of 2.5 ml of 41 per cent lead acetate in each tube. Mixed and filtered through Whatman No.42 filter paper. To 4.0 ml of filtrate, 1.0 ml aqueous solution of TBA (0.05 M) was added and the tubes were placed in a water bath at 40°C for 30-40 min. Refiltered the mixture through Whatman No.42 filter paper, in case, if observed any precipitate. The blank was prepared in the same way substituting distilled water for beverage sample. The absorbance (OD) was measured at 443 nm in a spectrophotometer (Spectronic-20, Bausch and Lomb) against a blank. The standard curve (Fig.4.1) prepared by using standard HMF (Sigma)solutions was used for calculations.

Regression equation obtained for HMF determination was:

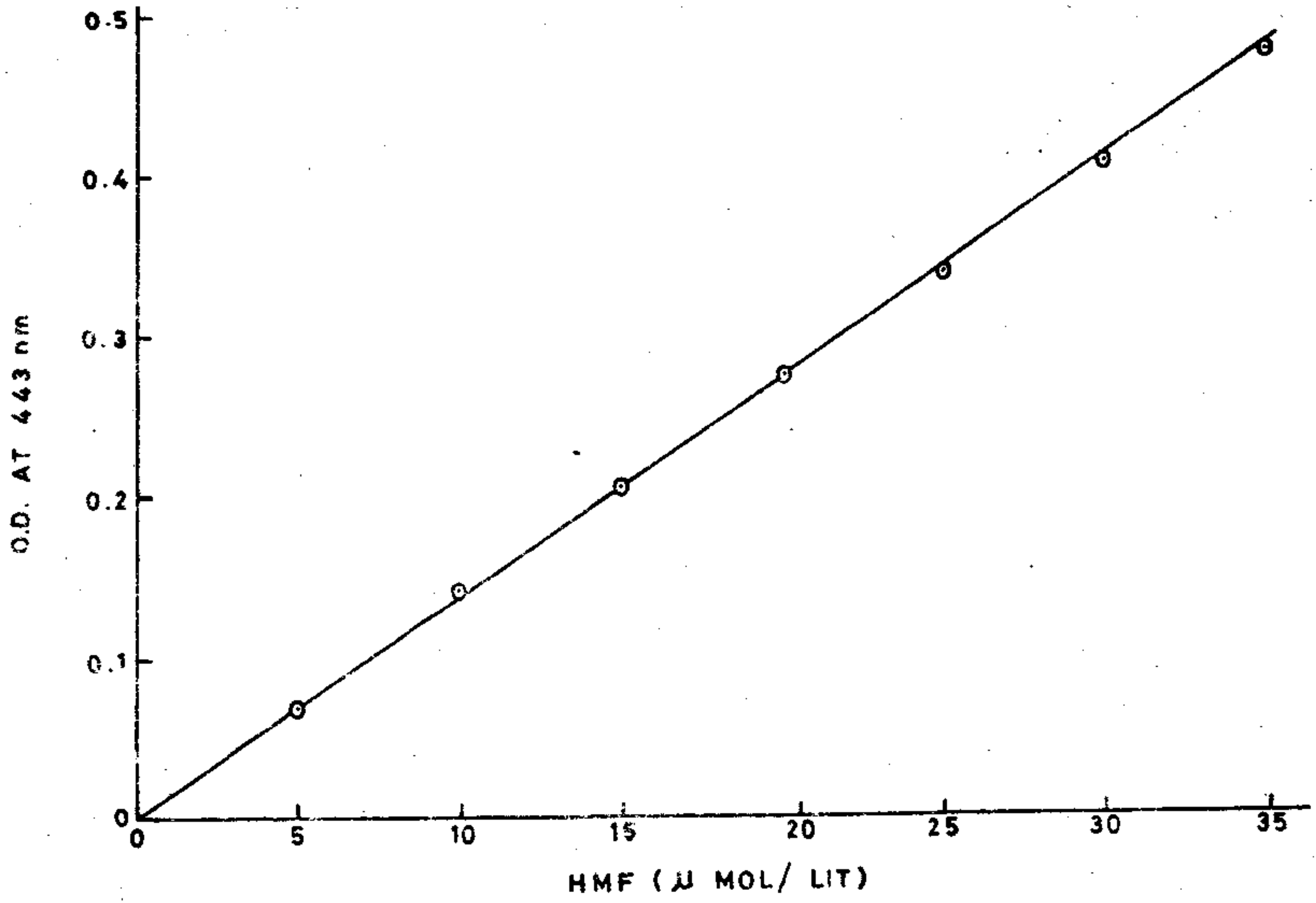

FIG. 4.1. STANDARD CURVE FOR TOTAL HMF

$$Y = 78.0342\,x - 1.2485$$

where,

x is absolute absorbance at 443 nm, and

y is HMF in µmol /lit of product

4.5.2.13 Total Spore Count

a) Sampling:

The samples of acidified milk beverage were opened in a covered chamber sterilized by U.V. radiation. An aseptic environment was created during sampling. Dilutions were prepared using 9 ml dilution blank prepared with 0.9 per cent sodium chloride solution.

b) Spore count:

Total spore count was done according to the procedure described in IS: 4238 (1967) using Tryptone Dextrose Agar medium (pH 7.0) of the following composition.

Ingredients	g/lit
Tryptone	10.0
Yeast extract	3.0
Soluble starch	1.0
Glucose	1.0
Agar (Bact.grade)	8.0

4.6 SENSORY EVALUATION

The sensory evaluation of beverage samples was carried out by a panel of trained judges selected from Dairy Technology Division. Scoring of the samples was done on a 9-point hedonic scale (Annexure-I) as recommended by Peryam and Pilgrim (1957). The samples were evaluated for various selected sensory parameters like flavour, body and texture, colour and appearance, and overall acceptability during the process of product development and storage studies.

4.7 STATISTICAL ANALYSIS

The sensory evaluation scores and the data on physico-chemical properties of the acidified milk beverage samples at each step of development were statistically analysed for analysis of variance (ANOVA), linear regression and coefficient of correlation according to the procedure recommended by Snedecor and Cochran (1967).

4.8 CONSUMERS ACCEPTANCE

Consumers acceptability trial was conducted at the Institute's Milk Parlour. The beverage was served to a total of 530 consumers from different strata of the society. The consumers were asked to indicate their preference of acceptability or non-acceptability for the product by placing a tick mark on the proforma given in Annexure II.

4.9 COST OF PRODUCTION

In the present study, the cost of production of acidified milk beverage was calculated on the basis of assumptions made as follows.

a) A small scale beverage factory with the capacity of 1000 kg of milk per day, was considered.

b) The factory will work for 300 days in a year and operate for one shift (8 hours) per day.

c) The handling losses of 1 per cent of the total milk handled was assumed, during manufacturing and packaging of the beverage.

d) It was presumed that excess of fat to be removed in the form of cream (40%) and will be considered as by-product.

e) The cost of raw buffalo milk (6% fat and 9.0% SNF) was assumed at the rate of Rs.5.50 per litre.

f) The expenditure on account of raw materials, utilities, labour, refrigeration, laboratory expenses, etc. were calculated essentially on the average prevailing rates for each item.

g) Building, land and equipment cost was considered as lump-sum investment.

h) The depreciation on capital investment i.e. building, equipment, machinery etc. has been charged on standard rates. The interest on working expenses and capital investment was charged on 16.5 per cent basis as recommended by Government for small scale industries loan.

i) It was expected that equipments suggested for a plant, will be able to accommodate the future expansion of milk handling upto 2500 kg per day.

CHAPTER - 5

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

This chapter deals with the results obtained during present investigation on acidified milk beverage. The first phase of this study was concerned with the optimisation of the technological parameters for the production of acidified milk beverage (AMB) and the consumers acceptability study of the beverage. In the second phase, studies were conducted on the shelf life of the acidified milk beverage stored in two types of packaging materials. Finally, attempts have been made to work out the cost of production of AMB on a commercial scale. The results have been presented mostly in tabulated form, but whereever necessary, they were further interpreted with the help of suitable illustrations. The results have been discussed under different headings.

5.1 PROCESS DEVELOPMENT FOR THE MANUFACTURE OF ACIDIFIED MILK BEVERAGE

5.1.1 SELECTION OF pH-SUGAR COMBINATION AND TYPE OF ACID

5.1.1.1 Preliminary Laboratory Trials

With the intention of developing a directly acidified milk based beverage, preliminary studies were conducted using four different types of acid viz. citric, phosphoric, lactic and hydrochloric. The acceptability of acidified beverage is entirely dependent on the optimum matching of sweetness and sourness. This can be achieved by trying different levels of sugar and pH conditions. However, different acids give entirely different intensity of sourness perception at the same sugar and pH levels combination. Keeping these facts in mind, various combinations (Table 5.1) were tried to arrive at most acceptable level of sugar and pH in the product.

Samples were prepared from pasteurized toned milk (3% fat and 8.5% SNF) in which weighed quantity of sugar (14, 16, 18 and 20% on w/v of toned milk) was dissolved at 45°C. Mixture was chilled to 6°-8°C before acidification (to minimize protein coagulation) and pH was then adjusted to different levels, viz. 4.50, 4.25, 4.00, 3.75 and 3.50 using different acid solutions (Table 4.1). Acidified samples were then homogenized using 'Rannie' single stage homogenizer (pressure 25-35 atm, and temperature 6°-8°C) and given to the judges for sensory evaluation.

From the Table 5.1, it was observed that in case of citric acid based beverage, the product was found to be more acceptable to judges at pH 4.25 and 4.00, when the sugar levels ranged between 16 and 20 per cent, and also with 20 per cent sugar at pH 3.75. The product was slightly acceptable at 14 per cent sugar level with pH 4.25 and 4.00, and at 14 to 18 per cent sugar level with pH 3.75. The samples at pH 4.50 and 3.50 were not acceptable to the judges at all sugar levels.

Table 5.1: Effect of pH and sugar levels and types of acid on acceptability of flavour.

Acid	pH	Sugar level (% w/v of toned milk)			
		14	16	18	20
Citric	4.50	-	-	-	-
	4.25	+	++	++	++
	4.00	+	++	++	++
	3.75	+	+	+	++
	3.50	-	-	-	-
Phosphoric	4.50	-	-	-	-
	4.25	-	+	-	-
	4.00	+	++	++	+
	3.75	++	++	++	++
	3.50	+	++	++	++
Lactic	4.50	++	++	+	+
	4.25	++	++	++	++
	4.00	+	+	+	+
	3.75	-	-	-	-
	3.50	-	-	-	-
Hydrochloric	4.50	-	-	-	-
	4.25	-	-	-	-
	4.00	-	+	-	-
	3.75	-	-	-	-
	3.50	-	-	-	-

++ Acceptable ; + Slightly acceptable ;
\- Not acceptable

Acidified beverage samples prepared using phosphoric acid were found acceptable at all sugar levels with pH 3.75. At pH levels of 3.50 and 4.00, the product was found only slightly acceptable with 14 per cent sugar level, but it was acceptable between 16 - 20 per cent sugar at 4.00 pH. Beverage with pH 4.25 and 4.50, was not found acceptable to the judges at sugar levels ranging between 14 - 20 per cent.

In case of lactic acid based beverage, the trend for acceptability was mostly reverse of that noticed for phosphoric acid. The product with higher pH was liked more than that with lower pH. Beverage with pH 3.50 and 3.75 was not found acceptable at all sugar levels (14-20%) but at pH 4.25, it was found to be more acceptable at all sugar levels. The product with pH 4.50 was acceptable only at 14 and 16 per cent levels of sugar due to less intensity of sourness and slightly acceptable at 18 and 20 per cent sugar levels, while pH 4.00 gave slight acceptability to the product at sugar levels between 14 and 20 per cent.

However, in case of hydrochloric acid based beverage, none of the sample at all pH and sugar levels was found acceptable during preliminary studies. Generally, the product had strong pungent odour and taste of chlorine and hence, inspite of tartness and sweetness in the product, it was not found acceptable to the judges. Therefore, the use of hydrochloric acid was discontinued in subsequent trials.

It is obvious from the above results that pH and sugar levels are very much important factors in the acceptability of acidified beverages prepared using different types of acids. More detailed organoleptic studies were conducted subsequently for beverage samples which were found to be acceptable in preliminary trials when prepared with different types of acids.

5.1.1.2 Optimisation of pH and Sugar Level Using Citric Acid

During preliminary trials on using citric acid in the preparation of milk based directly acidified beverage, it was observed that the samples with pH levels of 3.75, 4.00 and 4.25 were found to be slightly acceptable to acceptable at different sugar levels. A detailed study on sensory acceptability based on flavour score only was carried out to optimise the pH and sugar level in the product. The flavour acceptability scores are presented in Table 5.2 and analysis of variance in Table 5.3.

It is evident from the Table 5.2 that the acceptability score of the product at pH 4.00 was highest (6.97) at 18 per cent sugar followed by an average score of 6.77, 6.57 and 6.33 at 20, 16 and 14 per cent sugar levels, respectively.

Table 5.2: Effect of different levels of pH and sugar on flavour score, using citric acid.

pH level	Sugar level (% w/v of toned milk)				
	14	16	18	20	Mean
4.25	6.17	6.47	6.73	6.67	6.51
4.00	6.33	6.57	6.97	6.77	6.66
3.75	6.03	6.20	6.20	6.37	6.20
Mean	6.18	6.39	6.63	6.60	

Table 5.3: ANOVA for pH and sugar levels with citric acid.

Source of variation	d.f	M.S.S.	F.Value	C.D.
Replicates	2	0.003616	0.25	
pH	2	0.655283	45.13**	0.102
Sugar	3	0.394818	27.19**	0.118
pH x Sugar	6	0.037869	2.61*	0.204
Error	22	0.014520		

* $P < 0.05$; ** $P < 0.01$

At pH 3.75, highest average flavour score (6.37) was obtained for product with 20 per cent sugar, whereas, at 16 to 18 per cent sugar, the scores remained at 6.20, but with 14 per cent sugar, the flavour score decreased to 6.03.

The product with 4.25 pH obtained maximum score of 6.73 at 18 per cent sugar followed by an average score of 6.67, 6.47 and 6.17 at 20, 16 and 14 per cent sugar levels, respectively.

Statistical analysis of flavour scores (Table 5.3) showed that there was a highly significant difference ($P < 0.01$) between the flavour scores due to pH as well as sugar levels. Higher average scores were obtained at pH 4.00 followed by samples having pH 4.25. The present study revealed that the beverage prepared using citric acid with a pH of 4.0 and 18 per cent sugar was most acceptable on the basis of flavour score.

5.1.1.3 Optimisation of pH and Sugar Level Using Phosphoric Acid

The results on flavour evaluation scores of acidified milk beverage prepared with phosphoric acid with three pH levels (4.0, 3.75 and 3.50) and four sugar levels (14, 16, 18 and 20%), are presented in Table 5.4 and its analysis of variance in Table 5.5.

The average flavour score was found to be highest (7.30) in case of samples with pH 3.75 and 18 per cent sugar followed by average scores of 7.10, 6.87 and

Table 5.4: Effect of different levels of pH and sugar on flavour score, using phosphoric acid.

pH level	Sugar level (% w/v of toned milk)				Mean
	14	16	18	20	
4.00	6.53	6.90	6.87	6.83	6.78
3.75	6.73	7.10	7.30	6.87	7.00
3.50	6.50	6.70	6.73	6.77	6.67
Mean	6.59	6.90	6.97	6.82	

Table 5.5: ANOVA for pH and sugar levels with phosphoric acid.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.003616	0.50	
pH	2	0.328616	45.34**	0.072
Sugar	3	0.243985	33.67**	0.083
pH x sugar	6	0.036757	5.07**	0.144
Error	22	0.007247		

** $P < 0.01$

6.73 with sugar levels of 16, 20 and 14 per cent, respectively at same pH level.

At pH 4.00 and 3.50, the flavour scores increased gradually from 6.53 tø 6.90, and 6.50 to 6.77, respectively, with the increase in sugar level from 14 to 20 per cent. The maximum average scores of 6.90 and 6.77 were obtained for the beverage samples prepared with 4.00 pH and 16 per cent sugar level, and pH 3.50 and 20 per cent sugar level, respectively.

Analysis of variance (Table 5.5) also showed remarkable effect of pH and sugar level on flavour scores ($P < 0.01$). Amongst 12 combinations, the product at pH 3.75 with 18 per cent sugar gave maximum average score of 7.30 (CD = 0.144) followed by an average score of 7.10 for pH 3.75 with 18 per cent sugar; 6.90 for pH 4.80 with 16 per cent sugar; whereas pH 3.50 with 14 per cent sugar gave least score (6.50) to the product. Hence the product prepared with phosphoric acid with the combination of pH 3.75 and 18 per cent sugar level was selected for further studies.

5.1.1.4 Optimisation of pH and sugar level using Lactic Acid

Preliminary investigations showed that (Table 5.1) acidified beverage prepared with lactic acid, may be acceptable in the pH range of 4.50 to 4.00 at sugar levels of 14, 16, 18 and 20 per cent. A detailed study was therefore, undertaken to arrive at the optimum level of pH and sugar, on the basis of flavour scores.

The results of the evaluation of flavour scores of different pH-sugar combinations are presented in Table 5.6. It was observed that higher average score of 7.17 was obtained at pH 4.25 with sugar level of 16 per cent followed by average scores of 6.83, 6.73 and 6.63 with sugar levels of 18, 14 and 20 per cent, respectively at the same pH.

At pH 4.5, the higher average score (6.90) was obtained with 16 per cent sugar in the product. Slightly less score of 6.87 was obtained with 14 per cent sugar. The scores declined further to 6.57 and 6.37 at 18 and 20 per cent sugar levels, respectively probably due to more sweetness of the product.

The product at pH 4.00 obtained lower scores (6.00 - 6.40) at all sugar levels as compared to the scores obtained for product at pH 4.50 and 4.25, due to excessive sourness of the product even at 20 per cent sugar level.

ANOVA (Table 5.7) showed that the flavour scores of the beverage were affected more with pH levels ($P < 0.01$) than the sugar levels ($P < 0.05$).

5.1.1.5 Conclusive Remarks

Flavour is not a single, sharply-defined sensation but the combination of many basic sensations.

Table 5.6: Effect of different levels of pH and sugar on flavour score using lactic acid.

pH level	Sugar level (% w/v of toned milk)				Mean
	14	16	18	20	
4.50	6.87	6.90	6.57	6.37	6.67
4.25	6.73	7.17	6.83	6.63	6.84
4.00	6.00	6.17	6.27	6.40	6.21
Mean	6.53	6.74	6.56	6.47	

Table 5.7: ANOVA for pH and sugar levels with lactic acid.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.002500	0.08	
pH	2	1.293333	39.79**	0.153
Sugar	3	0.127685	3.93*	0.176
pH x sugar	6	0.156296	4.81**	0.305
Error	22	0.032500		

* $P < 0.05$; ** $P < 0.01$

In the product like acidified milk beverage, the optimum combination of sourness and sweetness or acidity (or pH) and sugar combination are very important. The sourness is dependent upon the acidity. Different acids give different intensity of sourness perception at the same pH level. Hence effort was made to select best combination of pH and sugar level and type of acid for the development of most acceptable beverage.

Based on the flavour scores of acidified beverage prepared with citric, phosphoric and lactic acid with four levels of sugar, it is seen from results presented in Table 5.2, 5.4 and 5.6 that sample prepared with phosphoric acid at a pH of 3.75 and with 18 per cent sugar secured maximum average score of 7.30 closely followed by an average score of 7.17 for sample prepared with lactic acid with a pH 4.25 and sugar level of 16 per cent. Samples prepared with citric acid at pH 4.0 and with a sugar level of 18 per cent ~~had~~ secured an average score of 6.97 only. On the basis of this flavour scores obtained, acidified beverage prepared with phosphoric acid with 3.75 pH and 18 per cent sugar was selected for further investigation. Since the aim was to prepare acidified milk beverage with lowest possible pH, product prepared with phosphoric acid as acidifier was found to be more acceptable at pH 3.75 and hence this acid was finally selected to standardize the technological parameters for the preparation of directly acidified milk beverage.

Dodd and Gupte (1990) based on their study on sugar content of various soft drinks and beverages available in the market, reported that there were significant variations in sugar content of different beverages which ranged from 5.06 to 17.29 gm/100 gm. Total sugar content of fruit based beverages was found to be higher and it ranged between 11.35 to 17.30 per cent.

5.1.2 STABILIZATION OF ACIDIFIED MILK SYSTEM

Stability of protein in milk system is an important consideration in the development of acidified milk beverage. The protein system can be destabilized easily due to alteration in salt concentration or pH. To maintain stability of acidified milk protein system at sterilization temperature is therefore, a very difficult task. Review of literature had indicated that the protein system can be stabilized by using different types of stabilizers, singly or in combination. Several patented methods for stabilizing sour drinks made from milk or skim milk are available. In most cases it was observed that heat treatment was given upto the pasteurization temperature only. To achieve the stability at sterilization temperature in the acid food system, there is a need for further investigation and hence number of stabilizers were tried in the present study for improving the stability of proteins using the preparation of directly acidified sterilized milk based beverage.

5.1.2.1 Selection of Suitable Type of Stabilizer

a) Initial screening of stabilizers:

The laboratory trials were conducted to determine the suitability of certain selected stabilizers on the basis of visual observations and comments given by a panel of judges on viscosity and on protein stability. For this study samples were prepared from toned milk (3% fat and 8.5% SNF) with the addition of 18 per cent sugar and stabilizers at the rate of 0.3 per cent. Only in case of k-Carrageenan a lower concentration (i.e. 0.03%) was used since it produced highly viscous product at higher concentration. All samples were adjusted to pH 3.75 using phosphoric acid.

Samples were initially observed by judges for their viscosity and then heat treatments were given until coagulation occurred in the products. The results presented in Table 5.8 indicated that only pectin (Sigma and local grade) and CMC-sodium salt (Sigma: low and medium viscosity grade) gave a lower viscosity to the product on the basis of visual observation. Remaining all other stabilizers had caused viscosity to increase considerably as was evident from visual observations. During heat treatment to the product, the samples were observed to coagulate between 42 to 72°C. Amongst different samples, samples with pectin and CMC were observed to coagulate at slightly higher temperatures

Table 5.8: Effect of stabilizers on viscosity and protein stability (visual observations)

Sr. No.	Stabilizer	Concentration (% w/v of milk)	Comments on viscosity	Coagulation temp (°C)	Remarks
1.	Pectin (Sigma)	0.3	Less viscous	63	Selected
2.	Pectin (local)	0.3	Less viscous	64	Selected
3.	CMC-Na Salt (Sigma, low viscosity)	0.3	Less viscous	61	Selected
4.	CMC-Na Salt (Sigma, medium viscosity)	0.3	Less viscous	72	Selected
5.	CMC-Na Salt (local, medium to high viscosity)	0.3	Slightly more viscous	50	Not selected
6.	Guar gum (local MV 2/4)	0.3	Highly viscous	52	Not selected
7.	Guar gum (local MV 2/3)	0.3	Highly viscous	52	Not selected
8.	Locust bean gum (Sigma)	0.3	More viscous	59	Not selected
9.	K-Carrageenan (Sigma)	0.03	Too much viscous	50	Not selected
10.	Agar (Sigma)	0.3	Slightly more viscous	42	Not selected
11.	Pregelatinized potato starch (local)	0.3	Viscous	43	Not selected

(61 to 72^{o}C). Since it was observed that pectin and CMC at 0.3 per cent level did not increase the viscosity in the beverage considerably, it was decided to further increase the level of these two stabilizers in the beverage. It was also expected that further increase in the level of stabilizer would also contribute to the increase in stability of the protein system in the beverage. Hence higher levels of pectin and CMC were selected for further studies.

In case of other remaining stabilizers if they were to be considered, then their levels in the beverage should be considerably reduced to decrease the viscosity development in the product. Reduction in level of stabilizer might not improve the heat stability of the protein system as even at 0.3 per cent, the stability was observed to be poor. On the other hand if the level is increased beyond 0.3 per cent, the stabilizers may cause undesirable rise in viscosity in the product and it may not be accepted as beverage. Hence other stabilizers were not considered for further studies.

b) Screening of selected stabilizers:

From the results (Table 5.8) on suitability of different stabilizers for acidified milk system, it was noticed that both types of pectin and CMC did not show much increase in viscosity of the product as judged by visual observations and also showed that the product could be heated to a slightly higher temperature before coagulation occurred in the product. These two stabilizers were thus selected for further study in acidified milk system at three higher levels viz. 0.4, 0.8 and 1.2 per cent. The products with added stabilizers were now subjected to heat treatment at 110°C for 5 min in all cases. The products were judged visually for viscosity and stability.

It is evident from Table 5.9 that in case of both types of pectin, the viscosity of the product at 0.4 per cent level did not increase considerably as compared to what was noticed at 0.3 per cent level earlier. But at 0.8 and 1.2 per cent level, there was considerable increase in the viscosity. Milk samples with both types of pectins at all three levels were found to coagulate on heat treatment.

In case of CMC (low viscosity grade), the viscosity increased as the level of CMC was increased from 0.4 to 1.2 per cent. Samples with 0.4 and 0.8 per cent level of CMC coagulated on heat treatment. But with samples containing 1.2 per cent CMC (low viscosity grade), there was no complete coagulation as noticed in case of pectins but a few flakes could be noticed in the product.

In case of CMC (medium viscosity grade) also the viscosity was found to increase as the level of

Table 5.9: Effect of stabilizer levels on viscosity and stability (at sterilization temperature) of acidified milk beverage (visual observations).

Sr. No.	Stabilizer	0.4%		0.8%		1.2%	
		Viscosity	Stability	Viscosity	Stability	Viscosity	Stability
1.	Pectin (Sigma)	Low	Coagulated	More viscosity	Coagulated	Very high	Coagulated
2.	Pectin (local)	"	"	"	"	"	Coagulated
3.	CMC - Na Salt (Sigma) low viscosity	"	"	"	"	"	Slightly coagulated
4.	CMC - Na Salt (Sigma) medium viscosity	"	"	Moderately high viscosity	Slightly coagulated	"	Not coagulated

stabilizer was increased from 0.4 to 1.2 per cent. Samples with 0.4 per cent level of CMC were completely coagulated during heat treatment. Samples containing 0.8 per cent CMC though not completely coagulated on heating but a few flakes of coagulated particles could be noticed. In case of samples containing 1.2 per cent CMC, there was no coagulation in the product. These results thus indicated that pectins even upto level of 1.2 per cent were not suitable for stabilizing acidified milk system. CMC (low viscosity grade) also was not able to completely stabilize the system at 1.2 per cent level. The stability of acidified milk system improved as the level of CMC (medium viscosity grade) was increased from 0.8 to 1.2 per cent. Hence CMC (medium viscosity grade) was selected for further studies.

5.1.2.2 Optimisation of CMC Level

a) Effect of CMC level on viscosity and stability:

Earlier laboratory trials had indicated that CMC (Na-salt, medium viscosity grade, Sigma) at 1.2 per cent level was able to stabilize directly acidified milk beverage having a pH of 3.75. But at 0.8 per cent level, the product showed signs of coagulation during sterilization. In order to confirm the suitability of using CMC, further detailed studies were carried out using three levels (viz. 1.0, 1.2 and 1.4%) of CMC in directly acidified milk beverage.

From Table 5.10 it was seen that as the level of CMC was increased from 1.0 to 1.4 per cent, the viscosity of the acidified milk beverage at 20°C increased from an average value of 12.51 cP to 23.87 cP. After sterilization, samples with 1.0 per cent CMC showed visible signs of slight coagulation. A few flakes of smaller size of coagulated particles could be noticed in a few samples. Samples with 1.2 and 1.4 per cent level of CMC had no visual coagulation. The present study further confirmed that in acidified milk, CMC (medium viscosity grade) around 1.2 per cent level, stabilized the product against heat coagulation.

b) Selection of most optimum level of CMC:

The investigation on stabilization of acidified milk system with CMC has shown that at 1.0 per cent level, a few flakes were noticed in sterilized product, whereas coagulation did not occur in product containing 1.2 per cent level of CMC. In order to arrive at optimum level of CMC required, three levels of CMC (medium viscosity grade) in the narrow range (1.15, 1.20 and 1.25%) were studied in the product and the product after sterilization (110°C for 5 min) was evaluated for flavour, and body and texture score by a panel of judges and also the viscosity of the product (in cP at 20°C) was measured.

It is evident from Table 5.11, the viscosity of the product increased from 14.20 cP to 19.58 cP as the level of CMC was increased from 1.15 to 1.25 per cent.

Table 5.10: Effect of CMC levels on viscosity and protein stability of beverage.

CMC level (%)	Viscosity at 20°C (cP)*	Visual observation on protein stability after sterilization at 110°C/5 minute.
1.0	12.51	Few flakes with smaller size particle in few samples.
1.2	16.92	No flakes, no coagulation
1.4	23.87	No flakes, no coagulation

* Average of six samples

Table 5.11: Effect of CMC levels on flavour, body and texture, and protein stability of acidified beverage.

Characteristics*	CMC level (%)		
	1.15	1.20	1.25
Flavour (score)	6.53	6.30	6.15
Body and Texture (score)	6.40	5.95	4.98
Viscosity (cP) at 20°C	14.20	16.97	19.58
Protein stability (visual observation)	No coagulation	No coagulation	No coagulation

* Average of four replicates

The average flavour score of the product decreased from 6.53 to 6.15 as the level of CMC was increased from 1.15 to 1.25 per cent. Similarly the body and texture score also decreased from 6.40 to 4.98 with increase in CMC level. Under experimental conditions, a lower level of 1.15 per cent CMC was found to be more acceptable with product, also the product on sterilization did not show signs of coagulation and therefore, CMC (Na-salt, medium viscosity grade) at 1.15 per cent level was tried in all further trials.

It is believed that curdling of the milk is prevented by a complex that is formed between the casein and the acidic carboxyl groups of the CMC molecule (Hidalgo and Hansen, 1969). Shenkenberg et al. (1971) developed the formula for stabilized milk-orange juice beverage, containing 0.2 per cent CMC. CMC-Na salt have also been used in fruit flavoured milk beverage within the range of 4.2 to 6.0 per cent (Nishiyama, 1976; Nishiyama, 1982).

5.1.3 OPTIMISATION OF LEVEL OF MILK SOLIDS

Although carboxymethyl cellulose (medium viscosity grade) was found to be "the most suitable stabilizer" at 1.15 per cent (w/v of toned milk) level in the acidified milk beverage, the acceptability of the product in terms of flavour, and body and texture score were found to be 6.53 and 6.40 respectively (Table 5.11).

Many of the judges commented that the product had higher viscosity to be considered as a beverage. Therefore, it was considered necessary to dilute the milk to some extent to reduce the viscosity of the product. Toned milk was therefore, diluted with water in ratios (v/v) of 80:20, 70:30 and 60:40. The quantity of added sugar and acid solution were kept constant. Quantity of stabilizer to be added was reduced proportionately to the milk dilution. The samples were sterilized at 110^{o}C for 5 minute, and were subjected to viscosity measurement and sensory evaluation.

5.1.3.1 Effect of Milk Dilution on Viscosity

It is evident from Table 5.12 that the dilution of milk with water showed a decrease in viscosity of the beverage, considerably. As compared to average viscosity of 14.20 cP in undiluted sample, the viscosity reduced to 10.53 cP in samples diluted with 20 parts of water. Further dilution with 30 and 40 parts of water, the viscosity decreased to 8.67 and 7.62 cP, respectively.

Table 5.12: Viscosity of acidified milk beverage made with different ratio of toned milk and water.

Toned milk:Water ratio	Viscosity (cP)*
100 : 00	14.20
80 : 20	10.53
70 : 30	8.67
60 : 40	7.62

* Average of three replicates

5.1.3.2 Effect of Milk Dilution on Body and Texture, and Flavour Score

It was further observed (Table 5.13) that the dilution of toned milk with water, considerably increased the body and texture score of beverage from 6.40 (for undiluted milk) to 6.90 and 7.38 for diluted milk at 80:20 and 70:30 ratio. Further increase in dilution to 60:40 ratio reduced the body and texture score to 6.55. A small increase in average flavour score was also noticed in acidified milk beverage due to dilution of milk in the preparation of acidified beverage. The average flavour score was observed to increase to 6.57 for beverage prepared with 80:20 ratio of dilution, and further increased to 6.73 at 70:30 ratio of dilution. Further dilution of milk to 60:40 ratio, caused the average flavour score to decrease to 6.23 mostly due to more watery taste. On the basis of

Table 5.13: Body and texture, and flavour score of acidified milk beverage made with different ratio of toned milk and water.

Toned milk : water ratio	Score*	
	Body and texture	Flavour
100:00	6.40	6.53
80:20	6.90	6.57
70:30	7.35	6.73
60:40	6.55	6.23

* Average of three replicates

these results dilution ratio of 70:30 for milk and water finalised for the manufacture of acidified milk beverage.

Since the higher level of milk solids gave more viscous body to the acidic beverage, many workers have tried lower level of milk solids for the preparation of acidified beverages. A method for manufacture of acidified milk beverage with low milk solids-not-fat (i.e. 1.5 to 4.6%) had been claimed, which remained stable without precipitation and separation (Anon, 1979). Yasumatsu *et al*. (1980a) have reported of stabilized acidified skim milk beverage with a pH of 3.35 - 3.75 and a milk-solids-not-fat content of 0.7 to 1.5 per cent (w/w).

5.1.4 OPTIMISATION OF STABILIZING SALT

5.1.4.1 Effect of Stabilizing Salts on Flavour, and Body and Texture Score

Addition of certain salts like tri-sodium citrate and dibasic potassium phosphate are reported to improve sensory characteristics of acidified beverages. Preparation of acidic beverage with phosphoric acid at pH 3.75 using CMC at 1.15 per cent level as the stabilizing agent had shown that the body and texture scores obtained was observed to be 6.4 (Table 5.11). Further improvement in body and texture score was obtained with proper dilution of the product (sec. 5.1.3). In order to further improve the sensory scores, if possible, by addition of salts, tri-sodium citrate and dibasic potassium phosphate were tried at three levels and the sensory score of acidified beverage was evaluated. It is evident from Table 5.14 that flavour and body and texture scores could be improved by the addition of salts in comparison to control (without salts). When dibasic potassium phosphate was added to the beverage at the rate of 0.06 per cent, the average flavour score increased from 6.73 (control) to 6.90. But further increase in level of this salt to 0.09 and 0.12 per cent caused the average scores to decrease to 6.73 and 6.67, respectively. The body and texture also was found to

Table 5.14: Effect of stabilizing salts on flavour, ~~and~~ body and texture score.

Stabilizing salts	Concentration (%)	Flavour score	Body and texture score
Control	-	6.73	7.37
Trisodium citrate	0.06	6.93	7.47
	0.09	7.13	7.77
	0.12	6.70	7.20
Dibasic potassium phosphate	0.06	6.90	7.47
	0.09	6.73	7.40
	0.12	6.67	7.20

increase at 0.06 per cent level of dibasic potassium phosphate, in comparison to control. The average body and texture score increased from 7.37 (control) to 7.47

at 0.06 per cent level addition of salt. But further increase in level to 0.09 and 0.12 per cent, caused decrease in the average body and texture scores to 7.40 and 7.20, respectively.

The use of tri-sodium citrate at 0.06, 0.09 and 0.12 per cent resulted in increase in the average flavour score from 6.73 (control) to 6.93, 7.13 and 6.70, respectively. Flavour scores increased upto 0.09 per cent level of addition of tri-sodium citrate. Further increase in the salt, caused the flavour score to decrease. The body and texture scores also increased from average of 7.37 (control) to 7.47 and 7.77 in case of beverage samples with 0.06 and 0.09 per cent added trisodium citrate. Further increase in the level of citrate to 0.12 per cent caused the body and texture scores to decreased upto 7.20.

Analysis of variance (Table 5.15 and 5.16) revealed that level of stabilizing salts had significant effect ($P < 0.01$) both on flavour and body and texture scores. Types of stabilizing salt also had highly significant effect ($P < 0.01$) on flavour score (CD, 0.072) and moderately significant effect on ($P < 0.05$) body and texture scores. The interaction effect among levels and types of stabilizing salt had significant ($P < 0.01$) both on flavour, and on body and texture scores (CD, 0.077). Among these two salts tried, although addition of both had shown improvement in certain sensory scores, the

Table 5.15: ANUVA for change in flavour score.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.002900	0.43	
Level of salt (L)	3	0.096655	14.36**	0.102
Type of salt (T)	1	0.081637	12.13**	0.072
L x T	3	0.053900	8.01**	0.144
Error	14	0.006728		

** $P < 0.01$

Table 5.16: ANOVA for change in body and texture score.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.002975	0.38	
Level of salt (L)	3	0.1582	20.61**	0.108
Type of salt (T)	1	0.050433	6.57*	0.077
L x T	3	0.050411	6.57**	0.153
Error	14	0.00767 7		

* $P < 0.05$; ** $P < 0.01$

improvement observed was more in case of trisodium citrate. Trisodium citrate at 0.09 per cent level was, therefore, selected for further studies on acidified milk beverage.

The citrate and phosphates are considered good peptizing agents which increase the hydration of casein. They also help to impart stability to the mix during heat treatment and processing (Jenness and Patton, 1959).

Glahn (1982) reported that when the pH of sour milk drink is reduced below 3.5, stability is adversely affected, probably due to suppression of the dissociation of the carboxyl groups of the hydrocolloid. Decreasing ionic strength, as by dilution of the drink with water, imparts stability, stability of such diluted drink can be restored by addition of buffer salts.

Hawthorne et al. (1977) reported that the addition of 0.03 per cent tri-sodium citrate in the pasteurized carbonated pineapple flavoured beverage, improved taste, cloudiness and frothing capacity to the product.

5.1.5 OPTIMISATION OF ADDED FLAVOUR

The flavour of food is all important. The aroma and flavour of food, if they are enticing and satisfying to the consumer, the eating becomes more pleasurable.

5.1.5.1 Effect of Added Flavour on Acceptability of Beverage

Although the beverage made from diluted toned milk (70:30) was acceptable as such, various synthetic flavours were tried to make the product more appealing. The preliminary laboratory trials had indicated that various flavours like pineapple, lemon and strawberry were more acceptable with acidified milk beverage than other flavours like mango, sweet orange and raspberry. The three flavours viz. pineapple, lemon and strawberry were added at the rate of 0.5, 1.0 and 1.5 ml per litre of beverage and subjected to sensory evaluation.

The results presented in Table 5.17, showed that the beverage sample with added pineapple, lemon and strawberry flavours at the concentration of 0.5, 1.5 and 1.0 ml per litre, respectively, were found to be

Table 5.17: Effect of different added flavours on acceptability score of the beverage.

Flavour	Concentration (ml/lit. of beverage)			Mean
	0.5	1.0	1.5	
Pineapple	7.366	7.900	7.133	7.466
Lemon	7.166	7.333	7.566	7.355
Strawberry	7.133	7.766	7.333	7.411
Mean	7.222	7.666	7.344	

generally acceptable. However, beverage sample containing pineapple flavour at the rate of 1.0 ml per litre was found more preferable (maximum average score, 7.90) amongst all samples.

The analysis of variance (Table 5.18) also revealed that there was no significant difference between three types of added flavours, but there was a highly significant difference ($P < 0.01$) between their levels of concentration. The interaction among level of concentration and types of flavour also had significant ($P < 0.01$) effect on flavour score. The present study, thus indicated that pineapple flavour at the rate of 1.0 ml per litre of beverage was most acceptable in directly acidified milk beverage prepared with phosphoric acid at pH level of 3.75.

Table 5.18: ANOVA for effect of added flavour on acceptability score.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.004461	0.32	
Flavours (F)	2	0.027794	2.02	0.1172
Levels (L)	2	0.474461	34.51**	0.1172
Interaction (F x L)	4	0.212214	15.43**	0.2030
Error	16	0.013750		

** $P < 0.01$

Review of literature had shown that generally in acidified milk based drinks or beverages, only sour fruit flavours such as pineapple, strawberry and lemon were used in most of the studies conducted by several workers on the development of such type of beverages.

5.1.16 OPTIMISATION OF ADDED COLOUR

First impression of any food is its physical appearance. For this reason many food product manufacturers take great deal of trouble to make the product more attractive to look at. The correct attractive colour influences the consumer in his choice and acceptance of a food product. For example, several prominent orange beverages have lemon as a predominating flavour but because they are coloured orange, the consumer associates their flavour with orange. Besides matching the colour with flavours, the intensity of colour is also another factor to be considered. Too much or too less addition of colour may also greatly affect the acceptance of product on the basis of sensory scores.

5.1.6.1 Effect of Colour Level on Acceptability of Beverage

Although the beverage was found to be more acceptable with pineapple flavour at the rate of 1.0 ml per litre of beverage, an effort was made to make the product more appealing to the consumer by using suitable food grade colour. For this purpose 'lemon yellow' colour (1% solution) was added at the rate of 1.0, 2.0, 2.5 and 3.0 ml per litre of beverage. The prepared product was given to different people at random to get their preference for colour intensity in the product. In all 74 people responded to this evaluation. The frequency distribution of the colour preference by the consumer panel is given in Table 5.19.

Table 5.19: Frequency distribution of consumers acceptability of acidified milk beverage with different levels of added colour.

Level of colour solution (ml/lit)	Number of consumer	Per cent
1.0	9	12.16
2.0	16	21.62
2.5	34	45.95
3.0	15	20.27
Total	74	100.00

It was observed that lemon yellow colour at the rate of 2.5 ml (1% solution) per litre of beverage was found more suitable and acceptable by the majority of the consumer (45.95% of total consumer) and hence this level was selected for further studies.

5.1.7 SUGGESTED TECHNOLOGY FOR THE MANUFACTURE OF ACIDIFIED MILK BEVERAGE

Based on preceding investigations, a process for the manufacture of acidified milk beverage is suggested.

Various steps involved in the manufacture of directly acidified milk based beverage are delineated in Fig. 5.1. The suggested steps for the preparation of directly acidified milk beverage are as follows.

* Standardization of milk to 3 per cent fat and 8.5 per cent SNF (i.e. toned milk).
* Dilution of toned milk with water in 70:30 (milk: water) proportion to get 2.1 per cent fat and 5.95 per cent SNF.
* Warming of the diluted milk to 45°C.
* Addition of sugar, stabilizer and stabilizing salt (tri-sodium citrate) at the rate of 18 gm, 0.005 gm and 0.09 gm respectively, per 100 ml of diluted milk.
* Mixing and dissolving of different ingredients and heating the mixture to 70°-75°C.
* Filtering of mixture, if needed.
* Cooling the mixture to about 6°-8°C.
* Addition of phosphoric acid solution (approximately 2.25 N) at the rate of 8.0 ml per 100 ml of diluted milk with vigorous agitation and final adjustment of pH to 3.75 with diluted acid.
* Heating to 65°C.
* Addition of colour solution (1% solution of lemon yellow) at the rate of 2.5 ml per litre of beverage.
* Homogenization of the mixture at 2500 and 500 psi at first and second stage, respectively.

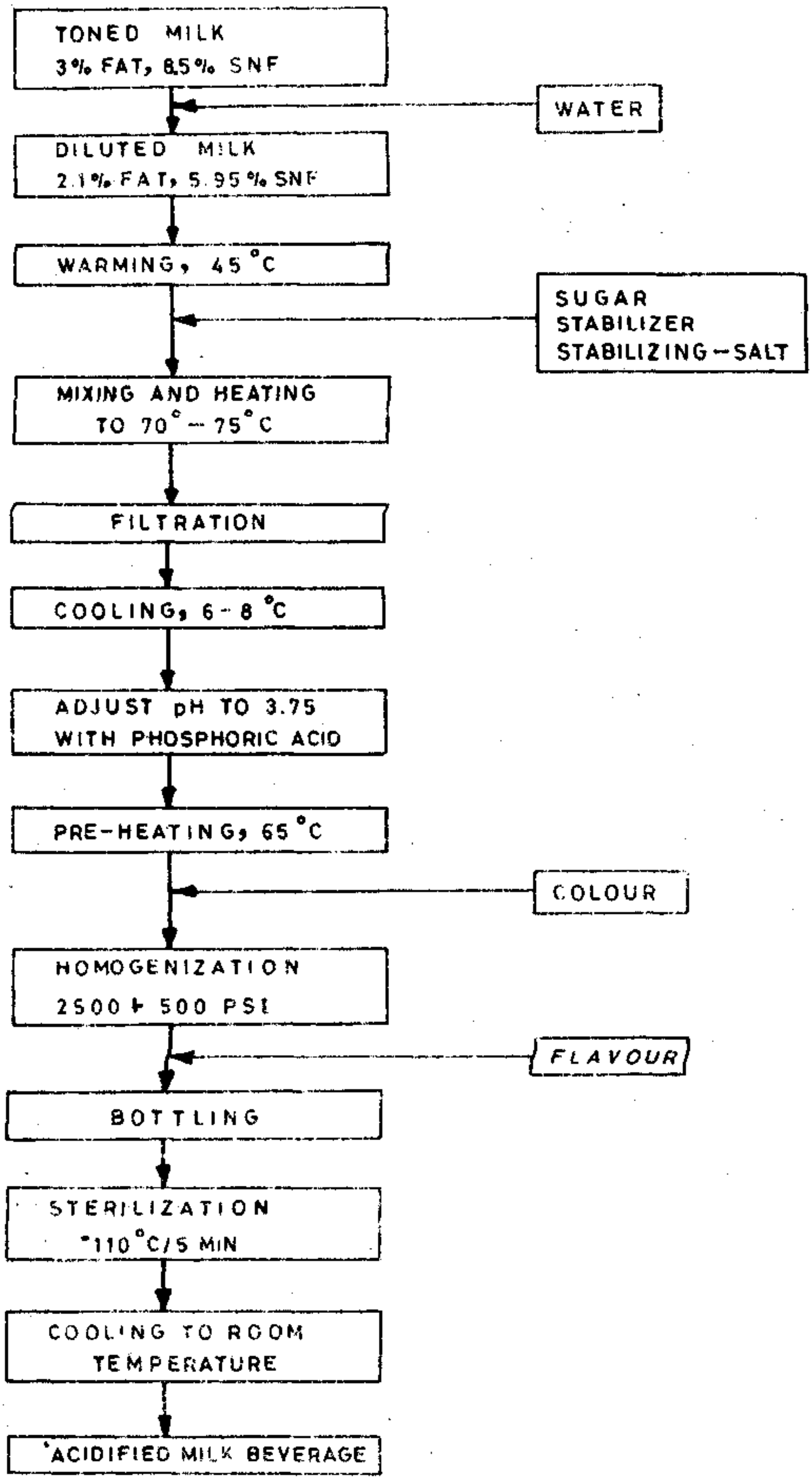

FIG.5.1. FLOW SHEET FOR THE MANUFACTURE OF ACIDIFIED MILK BEVERAGE

* Cooling the mixture at room temperature.
* Addition of pineapple flavour at the rate of 1 ml per litre of beverage with proper mixing.
* Filling in 200 ml sterilizable glass bottles and closing of the bottles with crown cork.
* Sterilization of the product at 110°C for 5 min in an autoclave.
* Cooling the product to room temperature.

Three batches of acidified milk beverages were prepared as per the standardized method and their physico-chemical characteristics and microbiological quality were evaluated. The physico-chemical characteristics and total spore count of the sterilized acidified milk beverage have been presented in Table 5.20. From this table it could be seen that the average total solids, fat and protein content were observed to be 22.04, 1.69 and 1.79 per cent, respectively. The average pH of the product was found to be 3.75. The viscosity was observed to be 8.701 cP at 20°C with the range of 8.520 to 8.910 cP. The average sediment content in the product expressed as ml/10 ml of beverage was 0.17. The initial reducing sugar level of the product was 3.33 per cent and free fatty acids content was 0.986 µ eq/ml. The TBA value (O.D), NPN content (%) and total HMF content (µ mol/lit) were observed to be 0.019, 0.0285 and 3.928, respectively. The product did not show any spore count.

Table 5.20: Physico-chemical characteristics and total spore count of fresh acidified milk beverage.

Sr. No.	Component	Range	Mean ± S.E.
1.	Total solids (%)	21.58-22.82	22.04 ± 0.39
2.	Fat (%)	1.65-1.73	1.69 ± 0.02
3.	Protein (%)	1.76-1.81	1.79 ± 0.013
4.	pH	3.74-3.76	3.75 ± 0.005
5.	Viscosity (cP)	8.520-8.910	8.701 ± 0.113
6.	Sediment (ml/10 ml)	0.15-0.20	0.17 ± 0.017
7.	Reducing sugar (%)	3.25-3.44	3.33 ± 0.05
8.	Free fatty acids (µ eq/ml)	0.952-1.020	0.986 ± 0.019
9.	Thio-barbituric acid value (O.D)	0.010-0.026	0.019 ± 0.0047
10.	Non-protein nitrogen (%)	0.0287-0.0294	0.0285 ± 0.00062
11.	Total Hydroxy methyl furfural (µ mol/lit)	3.667-4.058	3.928 ± 0.130
12.	Total spore count (per ml)	Nil	Nil

5.1.8 CONSUMER ACCEPTANCE FOR ACIDIFIED MILK BEVERAGE

Actual consumer response to a newly developed product is an important measure of the worthiness of the product. In order to elucidate the acceptability of the acidified milk beverage, it was necessary to expose it to a fairly large number of consumers and seek their opinion. The beverage, therefore, was subjected to a consumer acceptability test. The acidified beverage was prepared as per the standardized technique (5.17) and subjected to consumer acceptance test. The consumers were picked up at random and a prepared questionnaire (Annexure II) was given to them along with the product. The consumers were requested to evaluate the product and give their opinion of the product as per the instructions given in the questionnaire.

The frequency distribution of consumer acceptability is given in Table 5.21, 5.22 and 5.23. Out of 530 consumers, 103 (19.43%) consumers rated this product as excellent, whereas 211 (39.81%), 176 (33.21%), 34 (6.42%) and 6 (1.13%) consumer rated the product as very good, good, fair and poorly acceptable, respectively. None of the consumer found this product ~~under~~ non-acceptable grade. Only 6 out of 289 consumer in the age group of 25-50 rated this product as poorly aceeptable. Further it was found that the liking of the product differed with different age groups. Under all age group, 314 (59.24%)

consumer rated this product as very good and excellent (Table 5.21).

Table 5.21: Frequency distribution of consumer response (Acceptability) to acidified milk beverage according to age groups.

Acceptability	No.of consumers under different age groups				Total consumers	Overall per cent
	Under 16	16-25	25-50	Over 50		
Excellent	21	14	59	9	103	19.43
Very good	29	51	118	13	211	39.81
Good	16	60	88	12	176	33.21
Fair	-	14	18	2	34	6.42
Poor	-	-	6	-	6	1.13
Non-acceptable	-	-	-	-	-	-
Total	66	139	289	36	530	100.00

The acceptability scores were more or less similar among male and female consumer as evident from Table 5.22.

On the basis of acceptable scores given by different consumers, the average scores for each group of consumer was worked out and the results are presented in Table 5.23. For age group under 16, the average score comes to 9.07. This showed that the acceptability of the product in this age group was very good. The average

Table 5.22: Frequency distribution of beverage acceptability among male and female consumer.

Acceptability	Male		Female	
	Number of consumers	Per cent	Number of consumers	Per cent
Excellent	68	18.54	35	21.47
Very good	147	40.05	64	39.26
Good	126	34.33	50	30.68
Fair	22	5.99	12	7.36
Poor	4	1.09	2	1.23
Non-acceptable	-	-	-	-
Total	367	100.00	163	100.00

Table 5.23: Acceptability score of acidified milk beverage under different age groups

Attributes	Age group				Remarks
	Under 16	16-25	25-50	Over 50	
Number of consumer	66	139	289	36	530 (grand total)
Total score	599	1177	2518	317	4611 (grand total)
Average score	9.07	8.47	8.71	8.80	8.70 (overall average)
Acceptability remarks	Very good	Between good and very good	Nearer to very good	Nearer to very good	Nearer to very good

Maximum score: 10 Excellent; 9 Very good; 8 Good
7 Fair ; 6 Poor ; <6 Not acceptable

scores of 8.71 and 8.80 for consumer in the age group of 25-50 and over 50, respectively, indicated that the acceptability is nearer to very good. Even in case of consumer in the age group of 16-25, the acceptability (average score 8.47) was almost more than good. But the product was ~~liked~~ good to excellent in case of consumer under the age group of 16.

Based on the survey on 530 consumers of different age groups it may be presumed that this type of directly acidified milk based beverage will be acceptable by the majority of consumers once it is released for sale in the open market.

5.2 SHELF LIFE STUDIES ON ACIDIFIED MILK BEVERAGE

Acidified milk beverage (AMB) prepared as per the standardized method, was filled both in glass bottles (200 ml capacity) and metallised polyester pouches (200 ml capacity) and sterilized at 110^{0}C for 5 min. in a laboratory autoclave. The samples after cooling were divided into two lots. One lot was stored at 5^{0} $\pm$ 2^{0}C and the other at 30^{0} $\pm$ 1^{0}C. Samples packaged in glass bottles (B) and metallised polyester pouches (P) stored at 5^{0} $\pm$ 2^{0}C were designated as BR and PR, respectively. Whereas samples stored at 30^{0} $\pm$ 1^{0}C were designated as BC and PC, respectively, for the sake of convenience. The samples were examined for changes in their physico-chemical properties, microbiological quality and sensory characteristics at fifteen days intervals. The results obtained were statistically analysed to determine the relative merits of the packaging and storage conditions.

5.2.1 CHANGES IN CHEMICAL ATTRIBUTES

5.2.1.1 Free Fatty Acids (FFA)

If lipolytic activity occurs during the storage of sterilized acidified milk beverage, the content of FFA may increase and it may contribute to the development of off-flavour. The acidified milk beverage packaged in two types of packaging materials and stored at two different temperatures were analysed for changes in the FFA content during four months of storage. The results of investigation are presented in Table 5.24 and Fig 5.2, and analysis of variance in Table 5.25. It is evident from Table 5.24 that FFA content of AMB increased during the storage from an initial average value of 0.986 μ eq/ml to 1.836, 2.720, 1.700 and 2.425 μ eq/ml for BR, BC, PR and PC, respectively at the end of 120 days of storage. The maximum increase in FFA was noticed in BC followed by PC, BR and PR. Further it is evident from Fig. 5.2 that the rate of increase in FFA content was gradual upto 45 days of storage, both at 5^{o}C and 30^{o}C but the rate of increase in FFA was observed to be faster beyond 45 days of storage, the rate of increase being more at 30^{o}C

Table 5.24: Change in Free Fatty Acids (μ eq/ml) content of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	0.986	0.986	0.986	0.986	0.986
15	0.986	1.043	0.986	1.043	1.014
30	1.054	1.156	0.986	1.088	1.071
45	1.020	1.190	1.043	1.122	1.094
60	1.156	1.394	1.088	1.394	1.258
75	1.292	1.666	1.156	1.496	1.402
90	1.451	1.949	1.281	1.893	1.643
105	1.632	2.335	1.462	2.210	1.910
120	1.836	2.720	1.700	2.425	2.170
Mean	1.268	1.604	1.187	1.517	

of storage. Between the two types of packaging materials, the rate of increase in FFA content was observed to be higher in glass bottles than in metallised polyester pouches at both temperature of storage.

The analysis of variance (Table 5.25) showed that the increase in FFA content was significant due to period of storage ($P < 0.01$), packaging material ($P < 0.01$) and temperature of storage ($P < 0.01$), the effect of temperature being more pronounced than packaging materials. Further it was observed that the AMB could be stored for a period of 30 days and 15 days at 5°C and 30°C respectively,

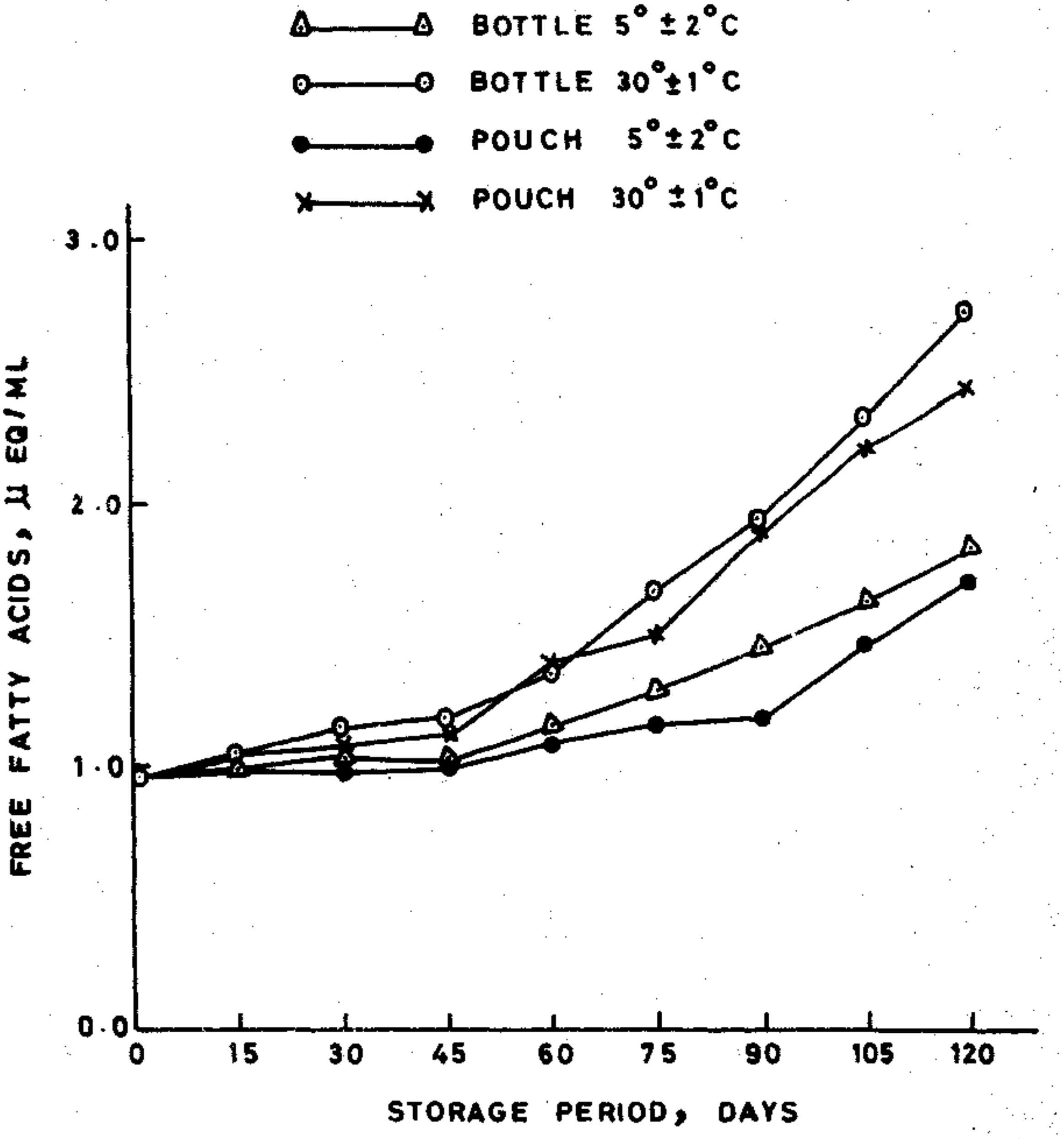

FIG. 5.2. CHANGE IN FREE FATTY ACIDS CONTENT OF ACIDIFIED MILK BEVERAGE DURING STORAGE

in both the packaging materials, before its FFA content increased significantly. The interaction between period and packaging material, and period and temperature had also noticed significant effect. Interaction effect due to package and temperature was not significant.

Table 5.25: ANOVA for change in FFA.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.006819	3.83*	
Storage periods (P)	8	2.181719	1221.26**	0.0344
Packages (PK)	1	0.189341	105.99**	0.0162
Temperatures (T)	1	2.995346	1676.70**	0.0162
(P x PK)	8	0.017816	9.97**	0.0487
(P x T)	8	0.267800	149.91**	0.0487
(PK x T)	1	0.000256	0.14	0.0229
(P x PK x T)	8	0.005037	2.82*	0.0688
Error	70	0.001787		

* $P < 0.05$; ** $P < 0.01$

A linear increase in FFA content in UHT milk upto a storage period of 6 months was reported by Malatje (1986). When stored at 4°C for 6 months, there was only a small increase (1.4 fold), which would not impair the organoleptic quality, but a 2.0 and 2.5 fold increase at 30°C and 40°C, respectively, which had a very unfavourable effect (Malatje, 1986).

At high storage temperature, a marked hydrolysis of milk fat can occur. When the BDI value (a measure of fat acidity, Bureau of Dairy Industries) exceeds 1.5, UHT milk is judged to be rancid. Driessen (1983) stored milk with lipolytic activity enhanced by the addition of Pseudomonas fluorescens at different temperatures. He reported that the threshold BDI value of 1.5 was exceeded after storage for 3 weeks at 20°C. Pantulu and Ramamurthy (1988) while studying the keeping quality of ghee packaged in metallised polyester pouches and glass bottles, observed that the rate of increase in FFA was slightly higher in glass bottles than in aluminium laminated pouches. No such information is available on the development of FFA in sterilized acidified milk beverage.

5.2.1.2 Thio-barbituric Acid (TBA value)

The results pertaining to TBA value of acidified milk beverage are presented in Table 5.26, Fig. 5.3 and its analysis of variance in Table 5.27. The TBA value (expressed as OD) of AMB increased during storage from 0.019 to 0.044, 0.166, 0.044 and 0.093 for samples BR, BC, PR and PC, respectively. The maximum increase was observed in samples stored in bottles at 30°C (BC), followed by PC, BR and PR. The ANOVA (Table 5.27) indicated that the period of storage, packaging materials and temp. of storage had significant effect ($P < 0.01$)

Table 5.26: Change in Thio-barbituric acid value (O.D.) of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	0.019	0.019	0.019	0.019	0.019
15	0.019	0.020	0.015	0.020	0.018
30	0.020	0.026	0.020	0.025	0.023
45	0.024	0.036	0.023	0.027	0.028
60	0.030	0.034	0.027	0.028	0.030
75	0.033	0.049	0.030	0.044	0.039
90	0.033	0.075	0.034	0.071	0.053
105	0.038	0.094	0.034	0.088	0.064
120	0.044	0.116	0.044	0.093	0.074
Mean	0.029	0.052	0.027	0.046	

on TBA value. The interaction effect due to period and package was not significant. However, effect of package and temperature was significant ($P < 0.05$). ANOVA also indicates that the effect of temperature is more pronounced than packaging materials and period of storage. Further it was observed that AMB could be stored for a period of 48, 44, 27 and 24 days respectively, in PR, BR, PC and BC before its TBA value increased significantly. The rate of increase in TBA value was also observed to be faster after two months of storage (Fig.5.3). A

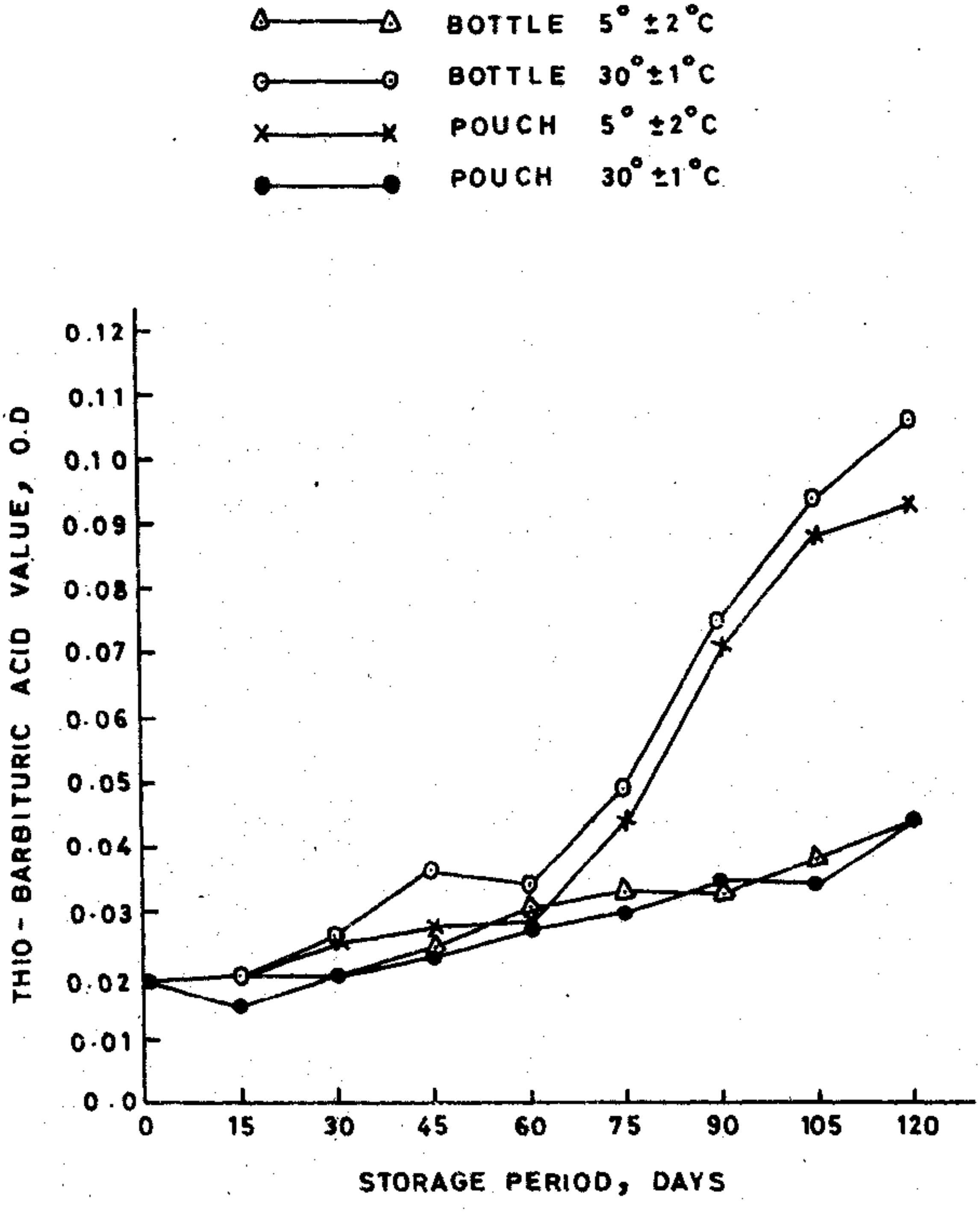

FIG. 5.3. CHANGE IN THIO- BARBITURIC ACID VALUE (O.D) OF ACIDIFIED MILK BEVERAGE DURING STORAGE

slight decrease noticed in TBA value (0.034) for sample stored in bottles at $30^{o}C$ for 60 days, could be due to sample to sample variation.

Table 5.27: ANOVA for change in TBA value.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.000067	2.13	
Storage periods (P)	8	0.005033	159.31**	0.0046
Packages (Pk)	1	0.000378	11.96**	0.0022
Temperatures (T)	1	0.011991	379.58**	0.0022
(P x Pk)	8	0.000037	1.19	0.0065
(P x T)	8	0.001720	54.45**	0.0065
(Pk x T)	1	0.000138	4.36*	0.0030
(P x Pk x T)	8	0.000045	1.44	0.0092
Error	70	0.000032		

* $P < 0.05$; ** $P < 0.01$

Several factors including temperature of storage and oxygen content of product are known to affect the rate of lipid oxidation (Parks, 1972). Wadsworth and Bassette (1985) observed that the UHT processed milk stored at higher temperature ($32^{o}C$) and with high initial oxygen concentration had higher TBA values than other samples. Singh and Patil (1989) observed that higher TBA values in buffalo UHT milk in comparison to cow UHT milk. The higher TBA value in buffalo UHT milk could either be due to difference in composition of fat or due to factors relating to processing or storage of milk. Moreover, buffalo milk as such has higher amount of polyunsaturated fatty acids with four or five double bonds as compared to cow milk and hence has lower autoxidative stability and shorter induction period (Rama Murthy and Narayanan, 1974). The rate of increase in TBA values was observed to be slightly higher in glass bottles than in metallised polyester pouches in case of ghee samples (Pantulu and Rama Murthy, 1988). In the present study also the increase in TBA value was observed to be slightly higher in glass bottles than in metallised polyester pouches.

5.2.1.3 <u>Non-protein Nitrogen (NPN)</u>

Change in non-protein nitrogen (NPN) content indicates the proteolysis of the product during storage. The NPN content of AMB was observed to increase from 0.0285 per cent to 0.0348, 0.0595, 0.0336 and 0.0565 per cent in BR, BC, PR and PC, respectively at the end of storage period (Table 5.28). The NPN content was observed to be slightly more in case of samples stored in bottles than in pouches (Fig. 5.4). The ANOVA (Table 5.29) indicated that the NPN content was significantly affected by the period of storage ($P < 0.01$), packaging materials ($P < 0.01$) and temperature of storage ($P < 0.01$). The effect of temperature was found to be more pronounced than period of storage and packaging material.

Table 5.20: Change in Non-protein nitrogen (%) content of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	0.0285	0.0285	0.0285	0.0285	0.0285
15	0.0278	0.0306	0.0282	0.0306	0.0293
30	0.0287	0.0341	0.0290	0.0336	0.0313
45	0.0299	0.0373	0.0296	0.0359	0.0332
60	0.0315	0.0399	0.0310	0.0385	0.0352
75	0.0324	0.0457	0.0315	0.0434	0.0383
90	0.0343	0.0506	0.0331	0.0569	0.0412
105	0.0343	0.0555	0.0331	0.0516	0.0436
120	0.0348	0.0595	0.0336	0.0565	0.0461
Mean	0.0313	0.0424	0.0309	0.0406	

The results obtained in this study are in agreement with those obtained by McKellar et al. (1984) who observed that the extent of proteolysis in directly heated UHT milk was closely correlated with length of storage at ambient temperature. Malatje (1986) also reported that the NPN content of UHT milk increased during storage at a rate dependent on the storage period. Sur and Joshi (1988) also reported gradual increase of NPN with increase of period of storage in UHT milk.

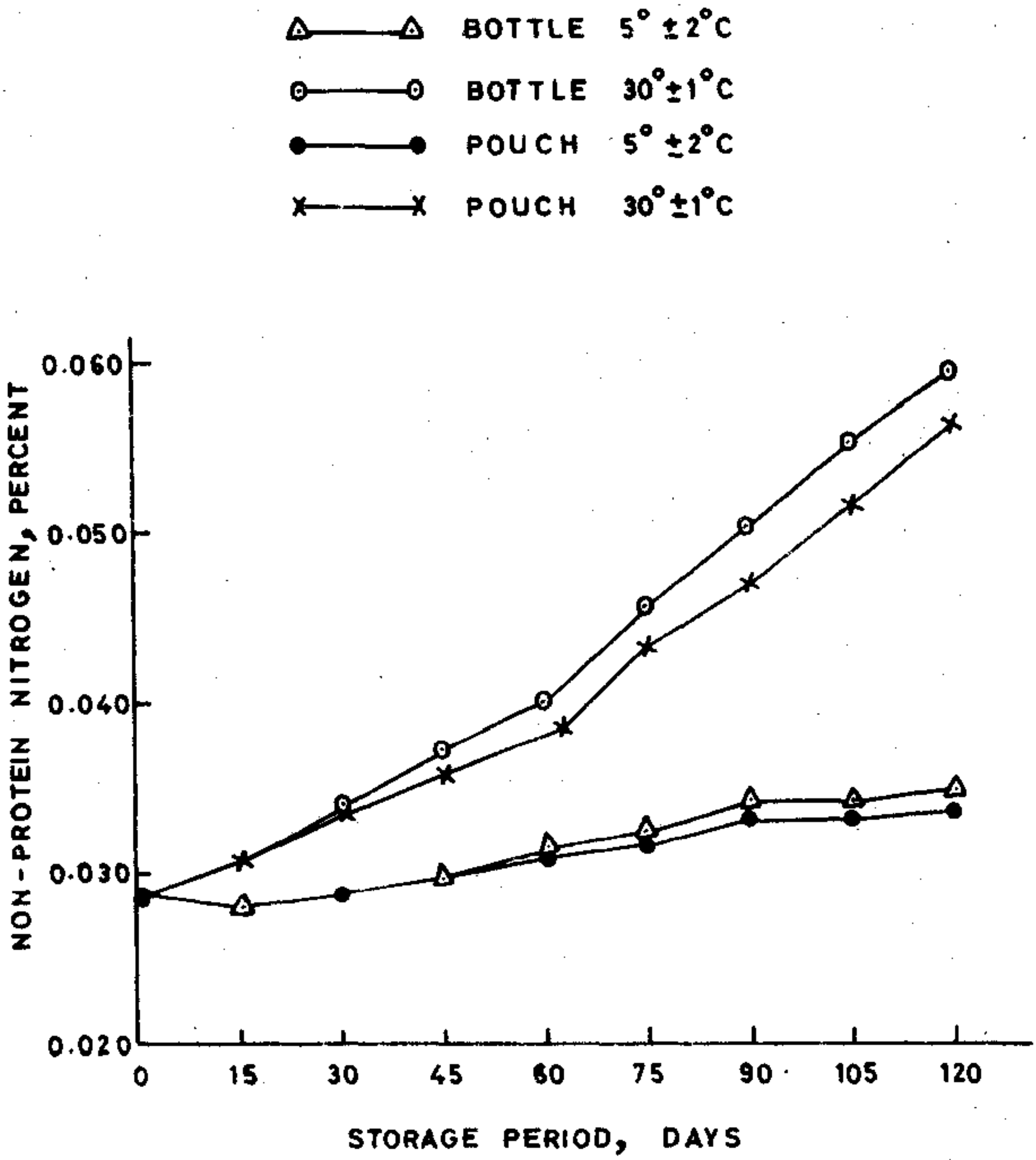

FIG. 5.4. CHANGE IN NON-PROTEIN NITROGEN CONTENT OF ACIDIFIED MILK BEVERAGE DURING STORAGE

Table 5.29: ANOVA for change in NPN.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.0000081	6.80**	
Storage periods (P)	8	0.00004858	406.97**	0.00089
Packages (Pk)	1	0.0000358	30.01**	0.00042
Temperatures (T)	1	0.0029245	2450.13**	0.00042
(P x Pk)	8	0.0000035	2.94**	0.00126
(P x T)	8	0.0001921	160.90**	0.00126
(Pk x T)	1	0.0000118	9.94**	0.00059
(P x Pk x T)	8	0.0000007	0.55	0.00178
Error	70	0.0000012		

** $P < 0.01$

Increase in non casein protein during storage may be due to the disintegration of casein-whey protein complex by proteases at higher temperature of storage. Such proteases either survive UHT treatment and reactivated subsequently during storage. Proteolytic break down of albumin nitrogenous fractions cause a decrease in its content resulting in the formation of peptides and NPN.

5.2.1.4 Reducing Sugar

When a sweetened, high acid drink is sterilized and subsequently stored, it is expected that the reducing sugar will increase during storage depending upon the temperature of storage. The results presented in Table

5.30 and Fig. 5.5 indicate the changes occuring in reducing sugar content during the storage of the product. As expected, the reducing sugar content of AMB increased from an initial average value of 3.33 per cent to 4.21, 7.89, 3.86, and 7.59 per cent for BR, BC, PR and PC,

Table 5.30: Change in Reducing sugar (%) content of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	3.33	3.33	3.33	3.33	3.33
15	3.33	3.57	3.33	3.56	3.45
30	3.36	4.00	3.37	3.79	3.63
45	3.37	3.49	3.40	4.15	3.85
60	3.52	4.74	3.45	4.29	4.00
75	3.61	5.50	3.56	5.38	4.51
90	3.80	5.99	3.64	5.79	4.80
105	3.98	7.03	3.77	6.43	5.30
120	4.21	7.89	3.86	7.59	5.89
Mean	3.61	5.17	3.52	4.92	

respectively at the end of 120 days of storage. The analysis of variance (Table 5.31) showed that the total reducing sugar content was significantly affected by period of storage ($P < 0.01$), packaging material ($P < 0.01$) and temperature of storage ($P < 0.01$); the effect of temperature being more pronounced than period of storage

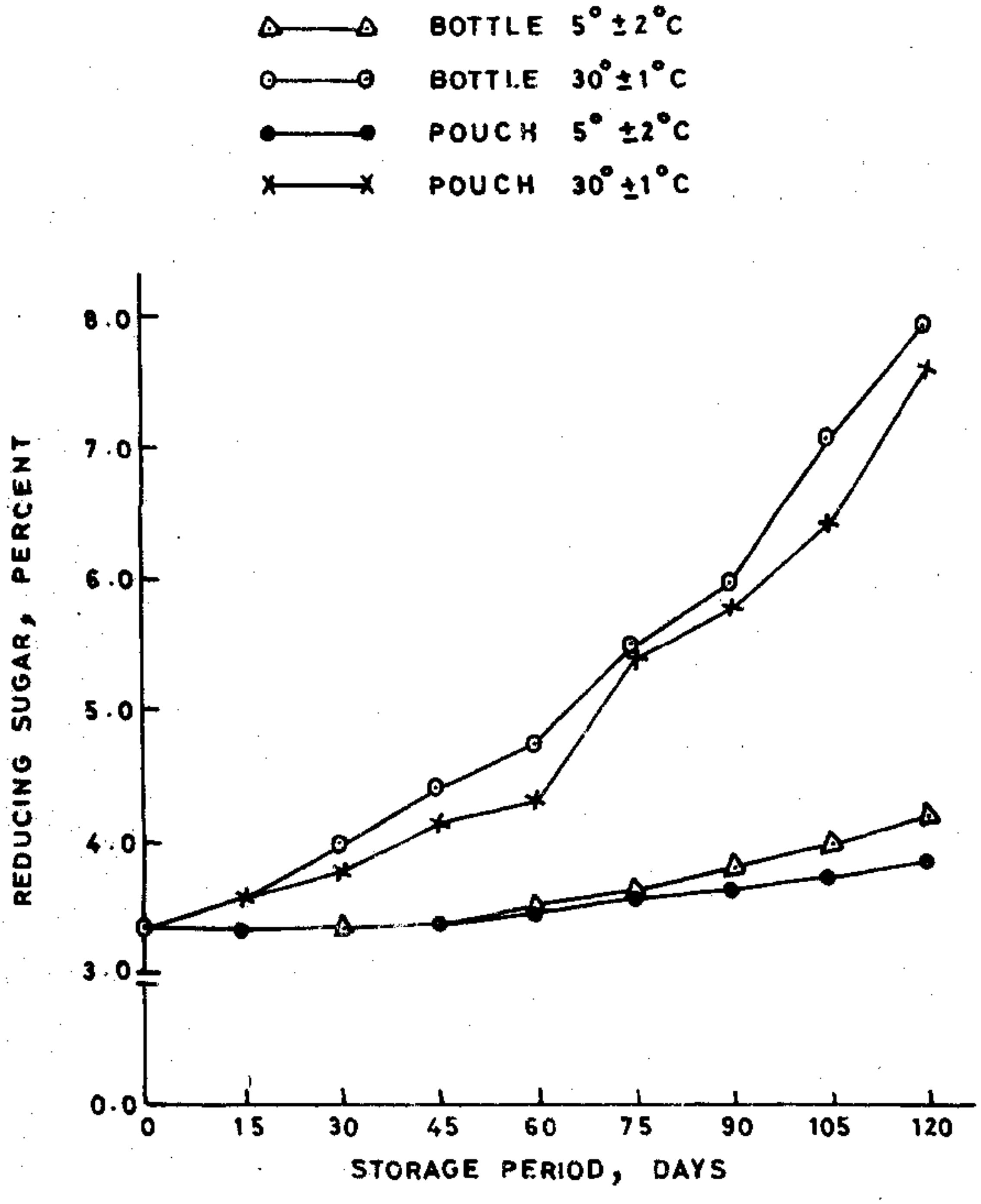

FIG. 5.5. CHANGE IN REDUCING SUGAR CONTENT OF ACIDIFIED MILK BEVERAGE DURING STORAGE

and packaging material. Further it was observed that the acidified milk beverage could be stored for a period of 55, 52, 7 and 7 days in PR, BR, PC and BC, respectively without significant changes in reducing sugar content. The present study indicated that the increase in reducing sugar content was more in samples stored in bottles than in metallised polyester pouches at both temperatures.

Table 5.31: ANOVA for change in Reducing sugar.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.1041	7.05**	
Storage periods (P)	8	9.3371	632.73**	0.099
Packages (Pk)	1	0.7617	51.62**	0.047
Temperatures (T)	1	59.1852	4010.71**	0.047
(P x Pk)	8	0.0578	3.92**	0.140
(P x T)	8	4.7258	320.24**	0.140
(Pk x T)	1	0.1736	11.76**	0.545
(P x Pk x T)	8	0.0248	1.68	0.198
Error	70	0.0148		

** P < 0.01

5.2.1.5 <u>Total 5-Hydroxy Methyl Furfural (HMF) Value</u>

5-Hydroxy methyl furfural (HMF) is one of the intermediate product of the Maillard reaction and its formation in milk is largely dependent on the type of heat treatment given to milk during processing (Patton, 1950).

HMF is also an index of browning reaction between free amino groups of protein and free aldehyde groups of reducing sugars present in any food system. Greater the extent of browning, greater is the HMF content (Patton, 1952; Hodge, 1953).

The total HMF content (Table 5.32 and Fig. 5.6) increased from an initial average value of 3.93 μ mol/lit to 7.15, 23.15, 6.50 and 18.57 μ mol/lit in BR, BC, PR and PC, respectively, at the end of

Table 5.32: Change in total HMF (μ mol/lit) content of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	3.93	3.93	3.93	3.93	3.93
15	4.24	6.03	4.19	5.12	4.90
30	4.42	8.04	4.24	6.97	5.92
45	4.71	9.62	4.45	8.19	6.74
60	5.54	11.16	5.02	9.99	7.93
75	5.96	13.84	5.46	12.41	9.42
90	6.28	16.75	5.67	14.70	10.85
105	6.89	19.87	6.22	15.42	12.11
120	7.15	23.15	6.50	18.57	13.85
Mean	5.46	12.49	5.07	10.59	

120 days of storage period. The analysis of variance

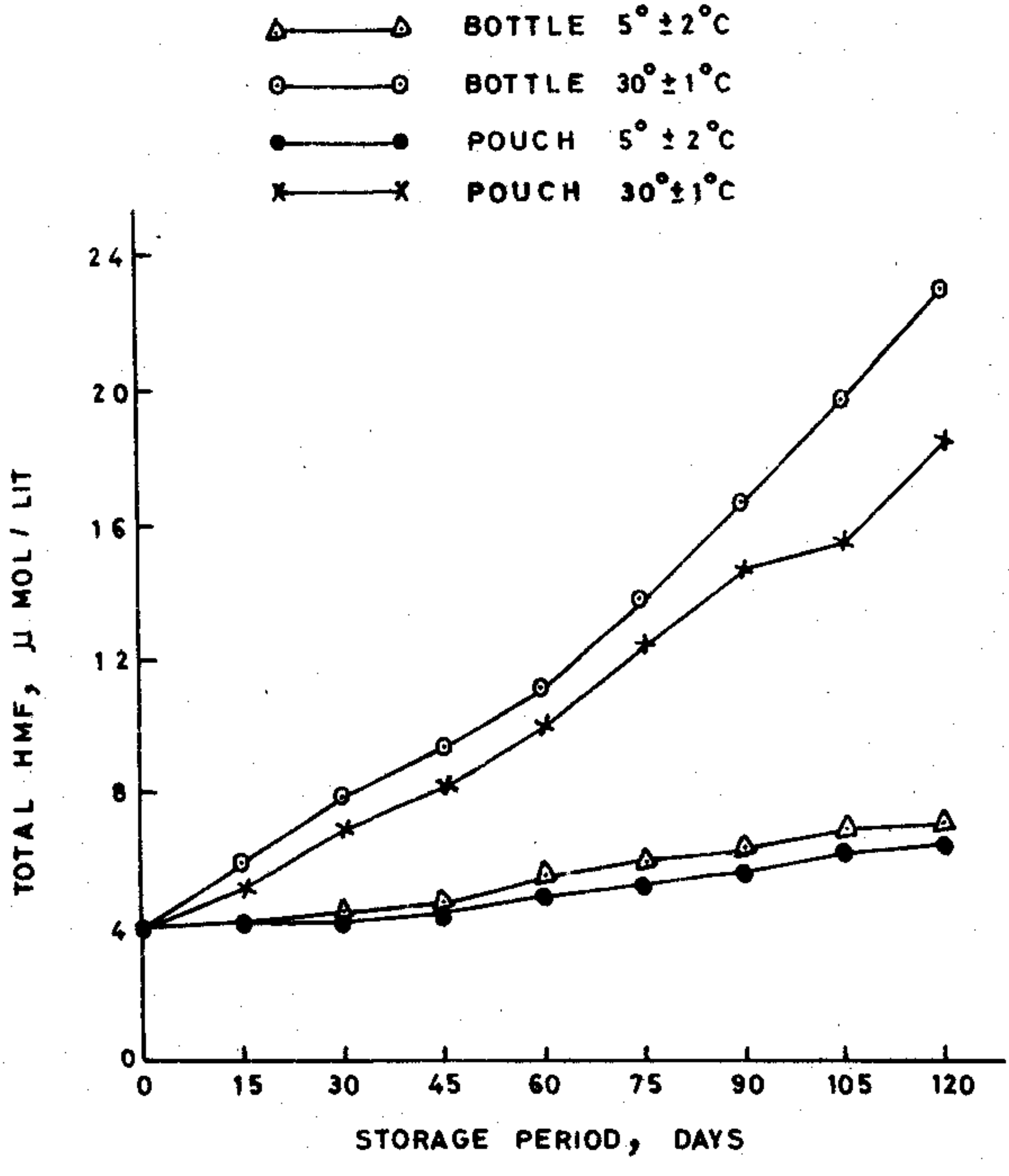

FIG. 5.6. CHANGE IN TOTAL 5-HYDROXY METHYL FURFURAL CONTENT OF ACIDIFIED MILK BEVERAGE DURING STORAGE

(Table 5.33) indicated that the HMF content was significantly affected by the period of storage ($P < 0.01$), packaging material ($P < 0.01$) and temperature of storage ($P < 0.01$). It was observed that the effect of temperature being more pronounced than period of storage and packaging materials. It was also observed that maximum amount of HMF was released in BC followed by PC, BR and PR.

Table 5.33: ANOVA for change in total HMF.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.002	0.01	
Storage periods (P)	8	137.673	752.29**	0.35
Packages (Pk)	1	35.132	191.97**	0.16
Temperatures (T)	1	1062.321	5804.87**	0.16
(P x Pk)	8	2.414	18.19**	0.49
(P x T)	8	65.482	357.82**	0.49
(Pk x T)	1	15.517	84.79**	0.23
(P x Pk x T)	8	1.429	7.81**	0.70
Error	70	0.183		

** $P < 0.01$

The HMF level in unheated milk is of importance in explaining the development of further HMF in heated milk. According to Klostermeyer <u>et al</u>. (1978), the HMF level in unheated whole milk was 11.1 μ mol/lit while for whole milk heated for 10 min. at 100°C, value was 19.83 µ mol/lit. Widely varying figures for total HMF content in commercially processed UHT milk have been reported (Töter, 1979; Mottar and Naudts, 1979; Renner and Dorguth, 1980; and Singh and Patil, 1989). Mottar et al. (1979), however, did not notice any rise in concentration of HMF during storage. They ascribed this to the possibility that HMF is lost by oxidation or other transformation during storage.

Varadaraju et al. (1988) also reported that during sterilization of buffalo milk in polypropylene pouches, the HMF value increased from 0.7 µ mol/lit in raw buffalo milk to 33 µ mol/lit after sterilization at 120°C for 4 minute. The value further increased to 4.6 µ mol/lit when sterilization was carried out at 120°C for 5 minute. Further they observed that browning of milk increased approximately three times during 30 days of storage at ambient temperature.

In their study, Fink and Kessler (1986) reported that the HMF value of UHT milk was observed to be 3.5 µ mol/lit immediately after processing. The flavour threshold values of HMF is about 16 µ mol/lit, so that the products of the Maillard reaction may lead to a sensory change when the UHT milk is held at 35°C or above.

Similar results were obtained by Malatje (1986) who found that after storing UHT milk at 30°C for 6 months, the HMF concentration approached the flavour threshold value and at 40^{o}C the threshold value was exceeded after about 10 weeks. In the present study, it was noticed that HMF value in AMB steadily increased with period of storage, both at refrigerated temperature (5^{o}C) and elevated storage temperature (30^{o}C). Rate of increase was observed to more in samples stored in bottles than in metallised polyester pouches.

5.2.1.6 pH

The data on changes in pH value in AMB (Table 5.34 and Fig. 5.7) showed that it decreased significantly after 45 and 60 days of storage in BC and PC respectively, while there was no significant change

Table 5.34: Change in pH of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	3.750	3.750	3.750	3.750	3.750
15	3.747	3.747	3.750	3.750	3.748
30	3.750	3.747	3.747	3.750	3.748
45	3.743	3.747	3.753	3.743	3.747
60	3.743	3.730	3.747	3.740	3.740
75	3.743	3.720	3.747	3.730	3.735
90	3.737	3.723	3.743	3.723	3.732
105	3.730	3.703	3.743	3.690	3.717
120	3.730	3.680	3.733	3.683	3.707
Mean	3.741	3.727	3.746	3.729	

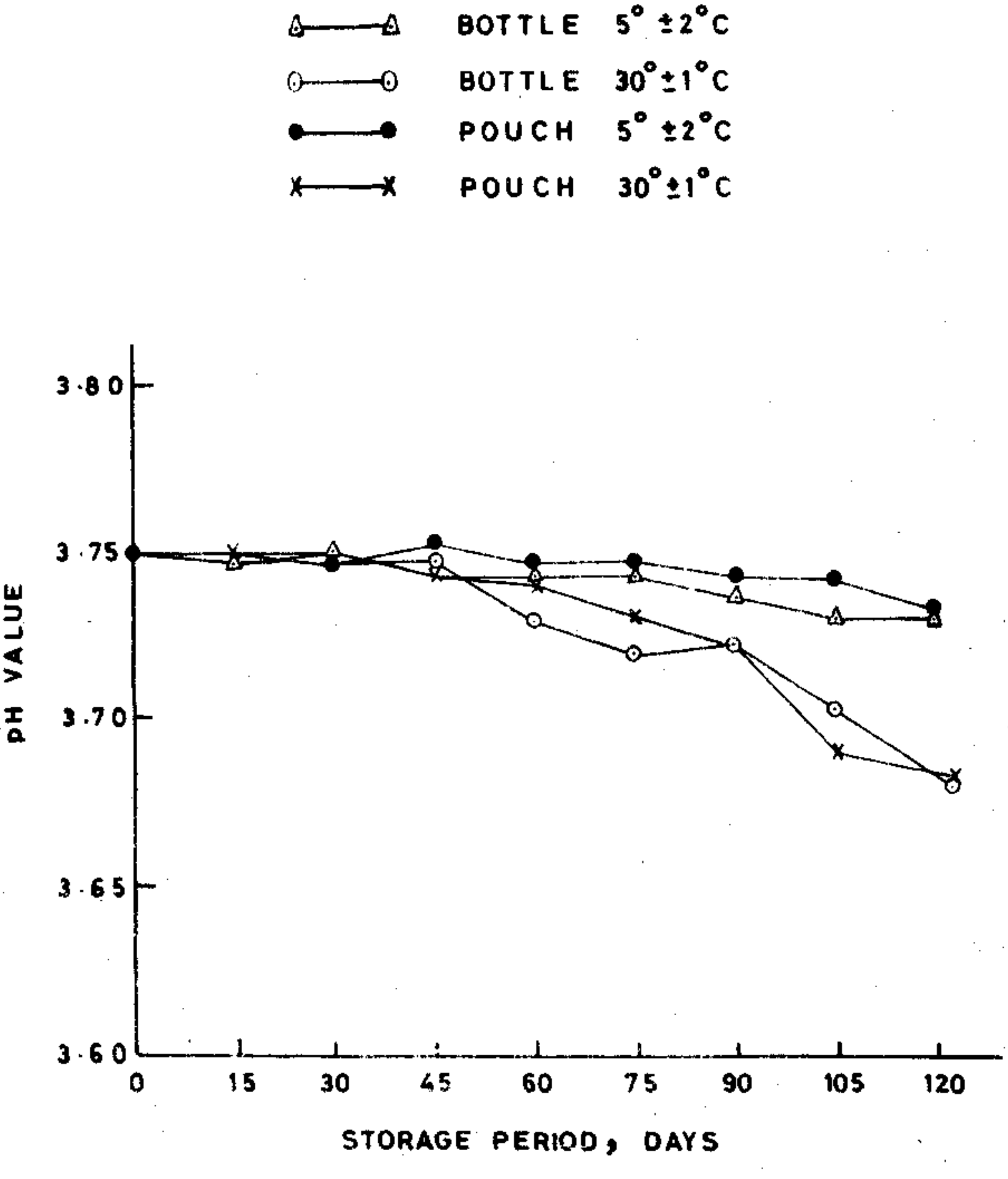

FIG. 5.7. CHANGE IN pH OF ACIDIFIED MILK BEVERAGE DURING STORAGE

in BR and PR almost upto 90 days (CD, 0.0187). The interaction studies had shown (Table 5.35) that the period of storage and temperature had significant affect on pH reduction in stored AMB. The interaction between packaging material and period of storage, and between packaging material and temperature of storage had no significant effect. Further, it was observed that the

Table 5.35: ANOVA for change in pH.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.000155	1.18	
Storage periods (P)	8	0.002825	21.45**	0.0093
Packages (Pk)	1	0.000229	1.74	0.0044
Temperatures (T)	1	0.006525	49.54**	0.0044
(P x Pk)	8	0.000021	0.16	0.0132
(P x T)	8	0.001018	7.73**	0.0132
(Pk x T)	1	0.000068	0.52	0.0062
(P x Pk x T)	8	0.000091	0.69	0.0187
Error	70	0.000132		

** $P < 0.01$

packaging material did not significantly affect the pH value, although the temperature and period of storage had significant ($P < 0.01$) effect. The simultaneous increase in HMF values (Table 5.32) also suggested the possibility of formation of various organic acids resulting fall in pH. Zadow and Chituta (1975) reported that a small reduction in pH value was observed only in UHT milk which had been stored for a long time.

5.2.2 CHANGES IN PHYSICAL ATTRIBUTES

5.2.2.1 Sediment

In most of the sterilized milk based beverages, the sediment formation has been reported to be affected by factors like initial quality of milk, processing conditions and storage conditions. The study on sediment formation in AMB showed that it increased from an initial average value of 0.17 ml/10 ml to 0.40, 0.55, 0.40 and 0.58 ml/10 ml of product in BR, BC, PR and PC respectively (Table 5.36). Though the sediment showed increasing trend

Table 5.36: Change in sediment (ml/10 ml) content of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	0.17	0.17	0.17	0.17	0.17
15	0.20	0.20	0.20	0.20	0.20
30	0.20	0.25	0.23	0.23	0.23
45	0.28	0.28	0.33	0.37	0.32
60	0.30	0.40	0.30	0.40	0.35
75	0.28	0.37	0.30	0.40	0.34
90	0.33	0.38	0.38	0.50	0.40
105	0.37	0.43	0.38	0.47	0.41
120	0.40	0.55	0.40	0.58	0.48
Mean	0.28	0.34	0.30	0.37	

during the storage period, the changes noticed were undulating (Fig. 5.8). At refrigerated temperature ($5^o \pm 2^oC$) upto 15 days of storage, the rate of sediment in BR and PR were found to be more or less the same.

The analysis of variance (Table 5.37) revealed that the period of storage as well as temperature of storage had pronounced effect ($P < 0.01$) on sedimentation

Table 5.37: ANOVA for change in sediment.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.000	0.01	
Storage periods (P)	8	0.133	42.12**	0.045
Packages (Pk)	1	0.017	5.96*	0.021
Temperatures (T)	1	0.104	36.68**	0.021
(P x Pk)	8	0.003	0.97	0.063
(P x T)	8	0.009	3.31**	0.063
(Pk x T)	1	0.001	0.40	0.030
(P x Pk x T)	8	0.001	0.27	0.089
Error	70	0.003		

* $P < 0.05$; ** $P < 0.01$

than the type of packaging material ($P < 0.05$). The interaction effect had also shown that only period of storage and temperature of storage (P x T) has significant effect ($P < 0.01$) on sediment formation, while period of storage and type of packages (P x Pk) and types of packages and temperature of storage (Pk x T) had no significant effect.

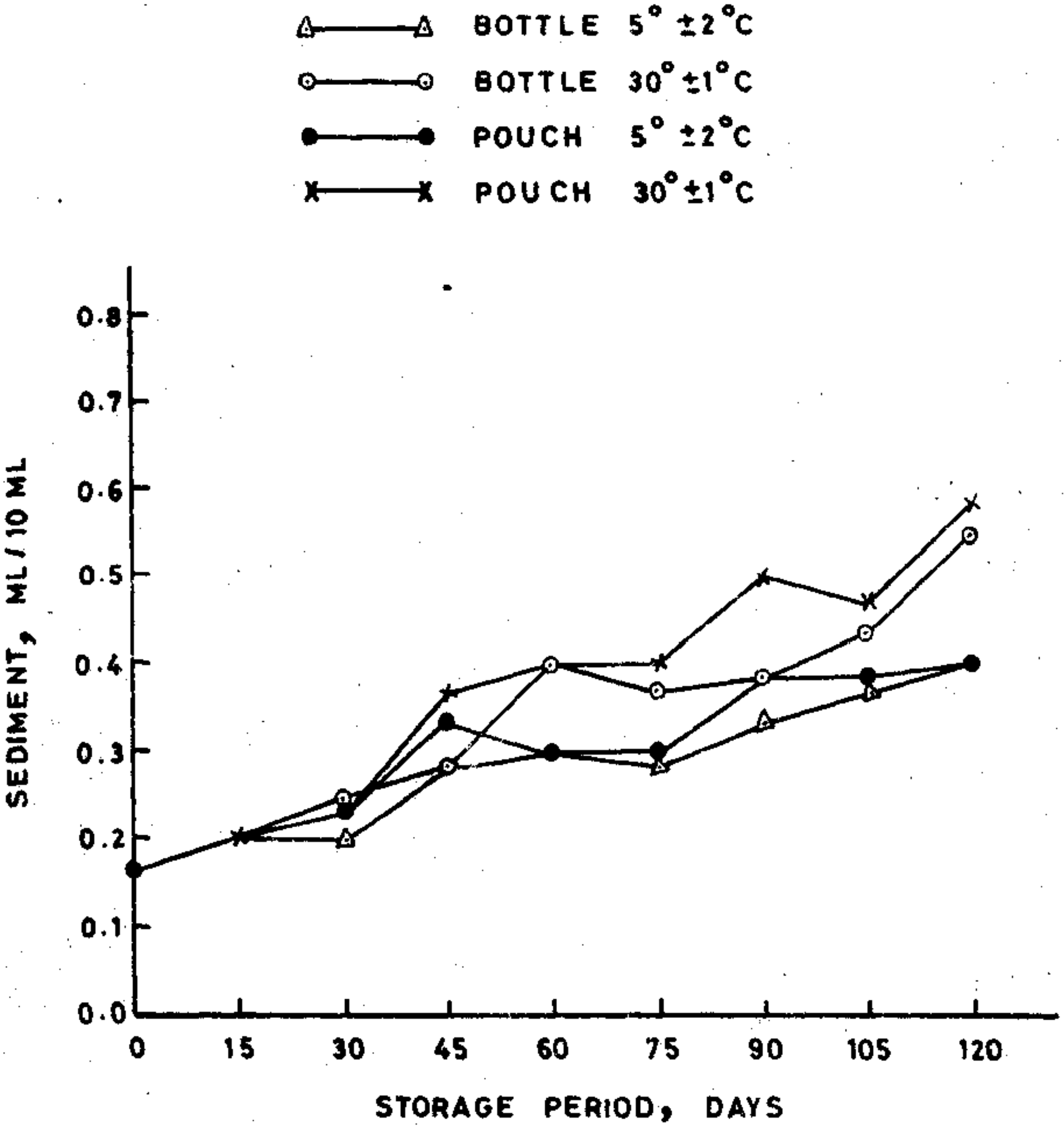

FIG. 5.8. CHANGE IN SEDIMENT CONTENT OF ACIDIFIED MILK BEVERAGE DURING STORAGE

Mehta (1980) observed that sedimentation in UHT milk was usually caused by denaturation of milk proteins or precipitation of the salts in milk. In acidified milk beverage also, the sedimentation could be due to denaturation of proteins or due to lack of complete complex formation between protein and stabilizer, and also due to precipitation of milk salts.

5.2.2.2 Viscosity

Viscosity is an important aspect of beverage acceptability. Changes occurring in physico-chemical properties of AMB during storage, may alter the viscosity of the product. This change in viscosity during storage, may have direct effect on the sensory quality of the product. Studies on the changes in viscosity of AMB during storage had shown (Table 5.38, Fig. 5.9) that the values increased from 8.701 cP to 9.610, 10.589, 9.440 and 10.294 cP for BR, BC, PR and PC respectively, after 120 days of storage. The increase in viscosity was observed to be higher at 30°C storage temperature than at 5°C with both types of packaging material. The statistical analysis (Table 5.39) had shown that the period of storage, temperature of storage and type of packaging materials have been significantly affected the viscosity of the beverage. Ashton (1965), and Harwalkar and Vreeman (1978) noticed rise in viscosity in stored UHT milk but only in later stages. Whereas, Blanc et. al. (1980) observed that during 16 weeks, viscosity decreased and a considerable increase was observed only after 32 weeks. In the present study, though the increase in viscosity was noticed in AMB, the maximum increment was only about 2 cP.

Table 5.38: Change in viscosity (cP) of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	8.701	8.701	8.701	8.701	8.701
15	8.753	8.823	8.702	8.752	8.757
30	8.840	8.914	8.779	8.868	8.850
45	8.931	9.181	8.818	9.064	8.990
60	9.107	9.381	8.989	9.178	9.166
75	9.204	9.607	9.050	9.353	9.304
90	9.270	9.821	9.260	9.600	9.488
105	9.594	10.251	9.383	9.707	9.734
120	9.610	10.589	9.440	10.294	9.983
Mean	9.112	9.474	9.014	9.280	

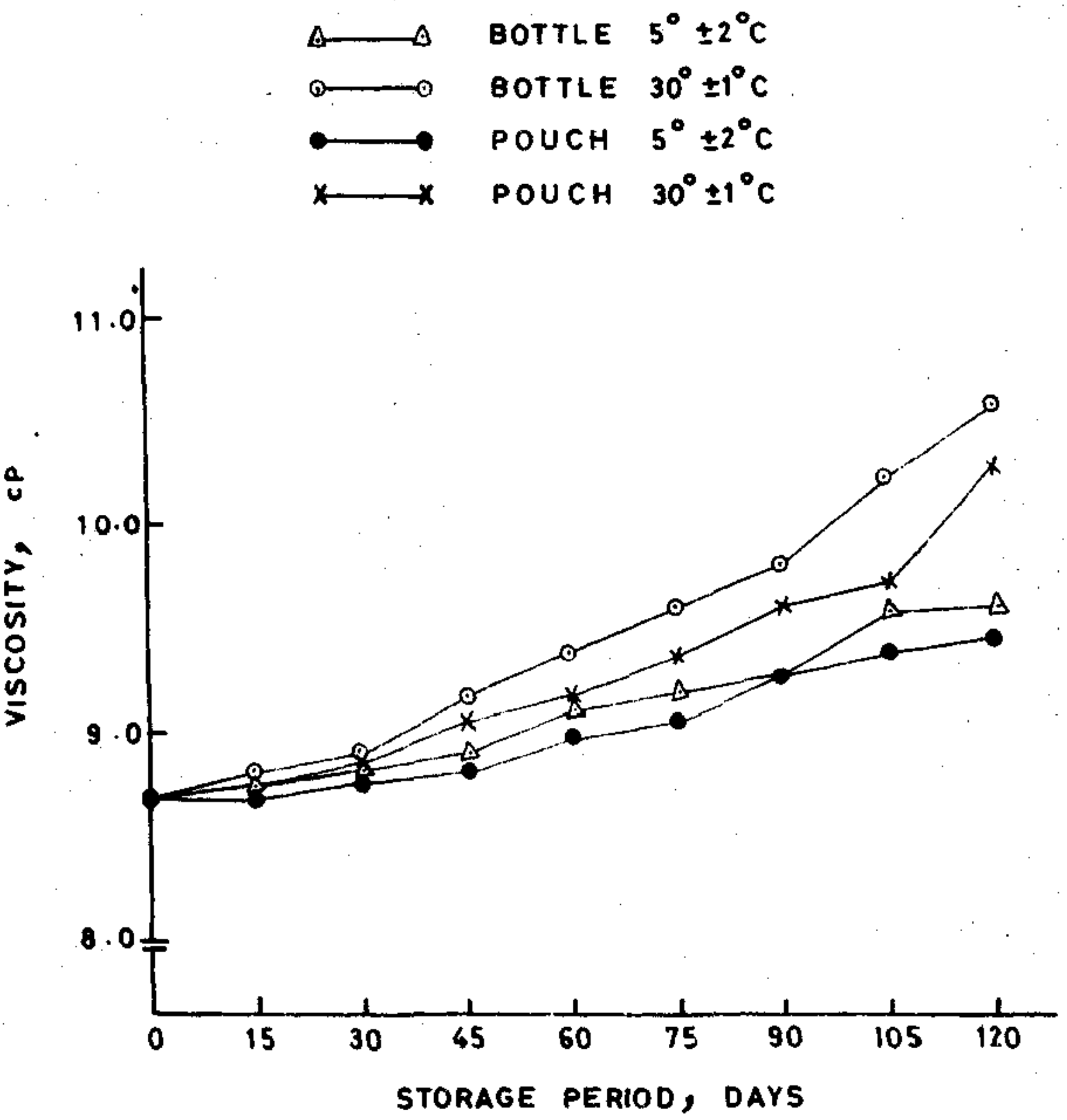

FIG. 5.9. CHANGE IN VISCOSITY OF ACIDIFIED MILK BEVERAGE DURING STORAGE

Table 5.39: ANOVA for change in viscosity.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.010352	0.50	
Storage periods (P)	8	2.396568	116.62**	0.1167
Packages (Pk)	1	0.579625	28.21**	0.0550
Temperatures (T)	1	2.660207	129.45**	0.0550
(P x Pk)	8	0.038869	1.89	0.1650
(P x T)	8	0.240703	11.71**	0.1650
(Pk x T)	1	0.061923	3.01	0.0778
(P x Pk x T)	8	0.009962	0.48	0.2334
Error	70	0.020549		

** $P < 0.01$

5.2.2.3 <u>Colour (Reflectance)</u>

Colour is the main sensory characteristics of milk and beverages which is immediately apparent and the only one which has been scientifically investigated on a practical basis (Blanc and Odet, 1981). The appearance and colour of milk as quoted by Hsu (1970) depend on the size gradient of the fat globule, distribution of milk proteins and the browning reactions.

Changes in colour during storage of AMB was measured in terms of per cent drop in reflectance and the results are presented in Table 5.40. It is evident from the table that in case of samples stored at 5°C, there was no drop in per cent reflectance upto 30 days of storage in both types of packaging materials. The per cent reflectance started decreasing beyond 45 days of storage. In case of BR, the reflectance dropped from 0.37 per cent at 45 days to 2.98 per cent at 120 days; while in case of PR the value dropped from 0.74 per cent to 2.98 per cent during the same period of storage. In case of higher storage temperature (30°C),

Table 5.40: Change in colour (% drop in reflectance*) of AMB during storage.

Storage period (days)	BR	BC	PR	PC
0	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00
30	0.00	0.74	0.00	0.74
45	0.37	1.49	0.74	1.11
60	1.11	2.61	1.49	2.24
75	1.11	2.98	1.50	2.60
90	1.86	3.35	2.23	2.98
105	2.24	4.48	2.60	3.35
120	2.98	5.22	2.98	4.47

* Average of three replicates

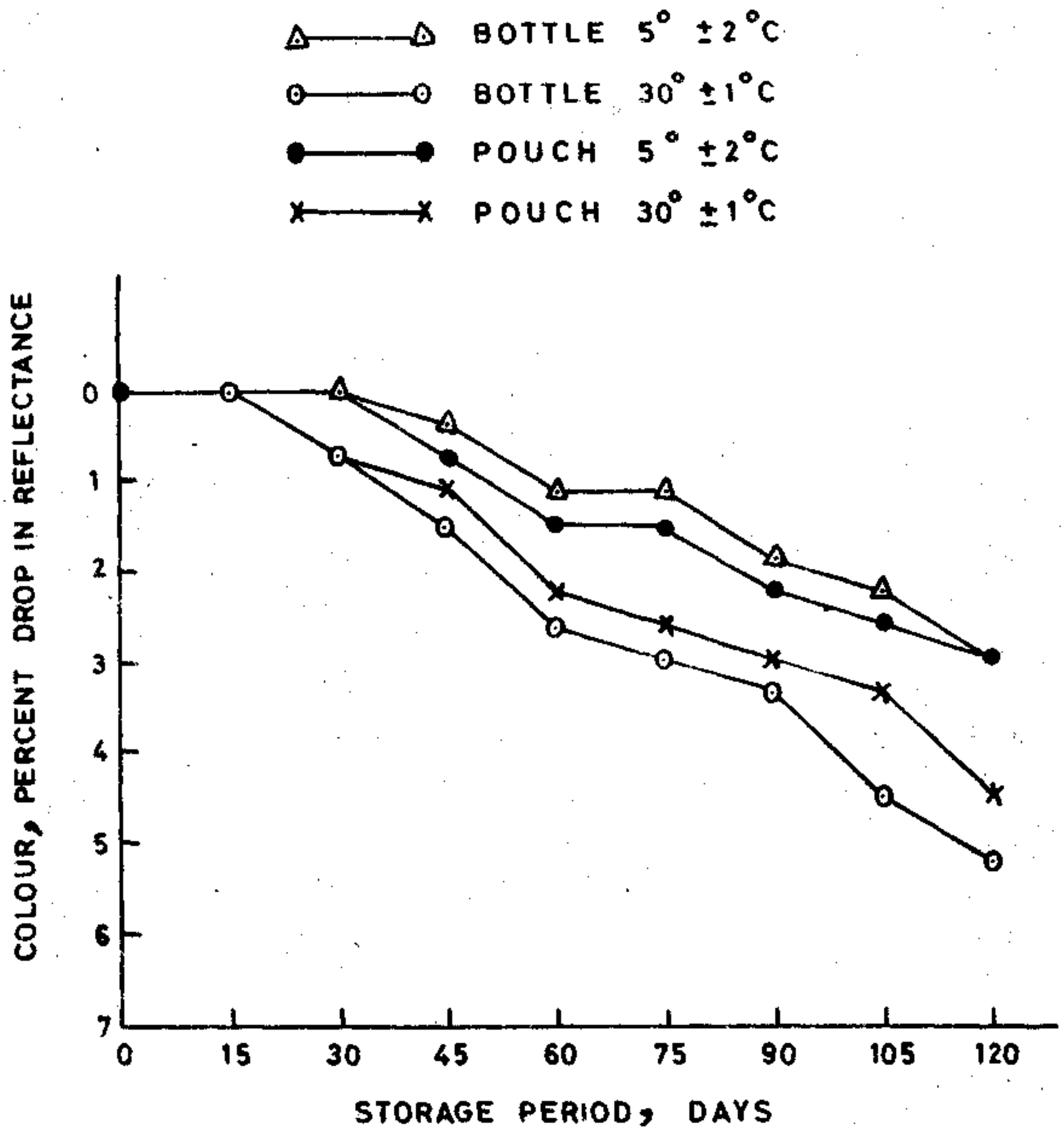

FIG. 5.10. CHANGE IN COLOUR (REFLECTANCE) OF ACIDIFIED MILK BEVERAGE DURING STORAGE

no change in reflectance was noticed till 15 days of storage in samples packaged in bottles and metallised polyester pouches. In case of BC, the value dropped from 0.74 per cent to 5.22 per cent between 30 and 120 days of storage. In case of PC, the value decreased from 0.74 per cent to 4.47 per cent during the same period. Graphical representation of change in colour recorded as per cent reflectance has been given in Fig. 5.10, which shows clearly that fall was quite considerable during storage period after one month.

According to Burton (1984), UHT processing causes only whitening in milk, while in-container sterilization causes whitening and browning together. The whitening, therefore, makes it difficult to separate and interprete the browning results.

5.2.3 CHANGE IN MICROBIOLOGICAL ATTRIBUTE

5.2.3.1 Total Spore Count

Resistance to heat is a common characteristic of bacteria of the genus *Bacillus* (aerobic) and of genus *Clostridium* (anaerobic). It is due to presence of spores. Milk, like other foods can be contaminated by *Bacillus* or *Clostridum* organisms. If any of the spores escape the effect of the heat treatment,they can cause problem in product during subsequent storage. From the available information, it is seen that clostridia germs rarely cause troubles in normal sterilized milk. *Bacillus* species on the other hand are common survivors and main cause of deterioration. Mostly thermophilic species are involved. The commercial techniques most widely used now-a-days, do not enable the spores of all species to be destroyed consistently. However, the number of Bacillus spores that survive in commercially sterilized product is reported toolow. It may vary between 0 to 10 per ml (Burton *et al.*, 1953).

Surviving spores can germinate rapidly in sterilized milk, if proper environment is created for their growth. However, the phase of latency which precede their germination can, in fact extend over a very long period in sterilized milk, many weeks or even three months. The alteration of the product due to multiplication of vegetative forms, their occur suddenly. The storagé time is therefore, of great importance in sterilized milk.

Total spore count of the AMB during storage was studied at different period and the results have been presented in Table 5.41. Initially, in all sterilized samples, total spore count was found to be nil, which showed that sterilization temperature and holding time used, were optimum for the sterilization of the product. After 60 days of storage, the total spore count was found to occur in samples in both the packaging materials. While analysing samples after 120 days of storage, the spore counts were found to be 4, 53, 12 and 44 numbers, respectively, in BR, BC, PR and PC. The increase in count indicate probably the surviving spores germinated due to change in environmental condition in the product during storage. According to Steinbuch (1974) the survivalibility of heat resistant bacterial spores during storage in acid conditions is affected by heat activation, pH, the acid used, the molarity of weak acids and other factors. (Blocher and Busta (1983) had reviewed and summerised the information on bacterial spore resistance to acid, and explained the possible mode of inhibition.) Generally acid conditions along with other environmental conditions, influence the growth

Table 5.41: Change in total spore count (per ml) of AMB during storage.

Storage period (days)	BR	BC	PR	PC
0	Nil	Nil	Nil	Nil
15	Nil	Nil	Nil	Nil
30	Nil	Nil	Nil	Nil
45	Nil	Nil	Nil	Nil
60	3	7	5	6
75	3	15	4	18
90	Nil	12	6	27
105	6	28	5	21
120	4	53	12	44

of microorganisms. So far, information on effect of reduced pH on bacterial spores are limited. Although study on various aspects of acid inhibition on microbial spores needs to be conducted. Erickson and Fabian (1942) reported that the thermal resistance of microorganisms in acid foods is a function of the chemical composition of the product and other factors, such as; the amount and type of sugar present, pH and type of acid used. The effect of organic acids on microorganisms is due to the toxicity of both the hydrogen ion and the undissociated molecule.

5.2.4 CHANGES IN SENSORY ATTRIBUTES

5.2.4.1 Flavour Score

The samples of AMB was evaluated for sensory scores at fifteen days interval. The initial average flavour score was 7.90 for freshly prepared AMB (Table 5.42). The flavour scores were observed to decrease progressively during storage period. The flavour scores decreased from an initial value of 7.90 to 6.60, 4.50, 6.30 and 4.00 for beverage stored in BR, BC, PR and PC, respectively, after 120 days of storage. ANOVA (Table 5.43) indicated that the period and temperature of storage had significant effect ($P < 0.01$) on flavour scores while type of packages used had no significant effect. The interaction effect also indicated that only period of storage and temperature (P x T) had significant effect ($P < 0.01$) on flavour scores. The flavour scores

Table 5.42: Change in flavour score of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	7.90	7.90	7.90	7.90	7.90
15	7.90	7.90	7.90	7.77	7.87
30	7.87	7.90	7.80	7.67	7.80
45	7.77	7.33	7.33	7.40	7.56
60	7.50	7.00	7.50	7.00	7.25
75	7.00	6.50	7.40	6.50	6.85
90	6.90	5.50	7.00	5.20	6.15
105	6.50	5.17	6.60	5.00	5.82
120	6.60	4.50	6.30	4.00	5.35
Mean	7.33	6.63	7.35	6.49	

Table 5.43: ANOVA for change in flavour score.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.014	0.15	
Storage periods (P)	8	11.151	124.71**	0.243
Packages (Pk)	1	0.095	1.07	0.114
Temperatures (T)	1	16.178	180.93**	0.114
(P x Pk)	8	0.077	0.87	0.343
(P x T)	8	1.895	21.19**	0.343
(Pk x T)	1	0.179	2.00	0.162
(P x Pk x T)	8	0.023	0.26	0.486
Error	70	0.089		

** $P < 0.01$

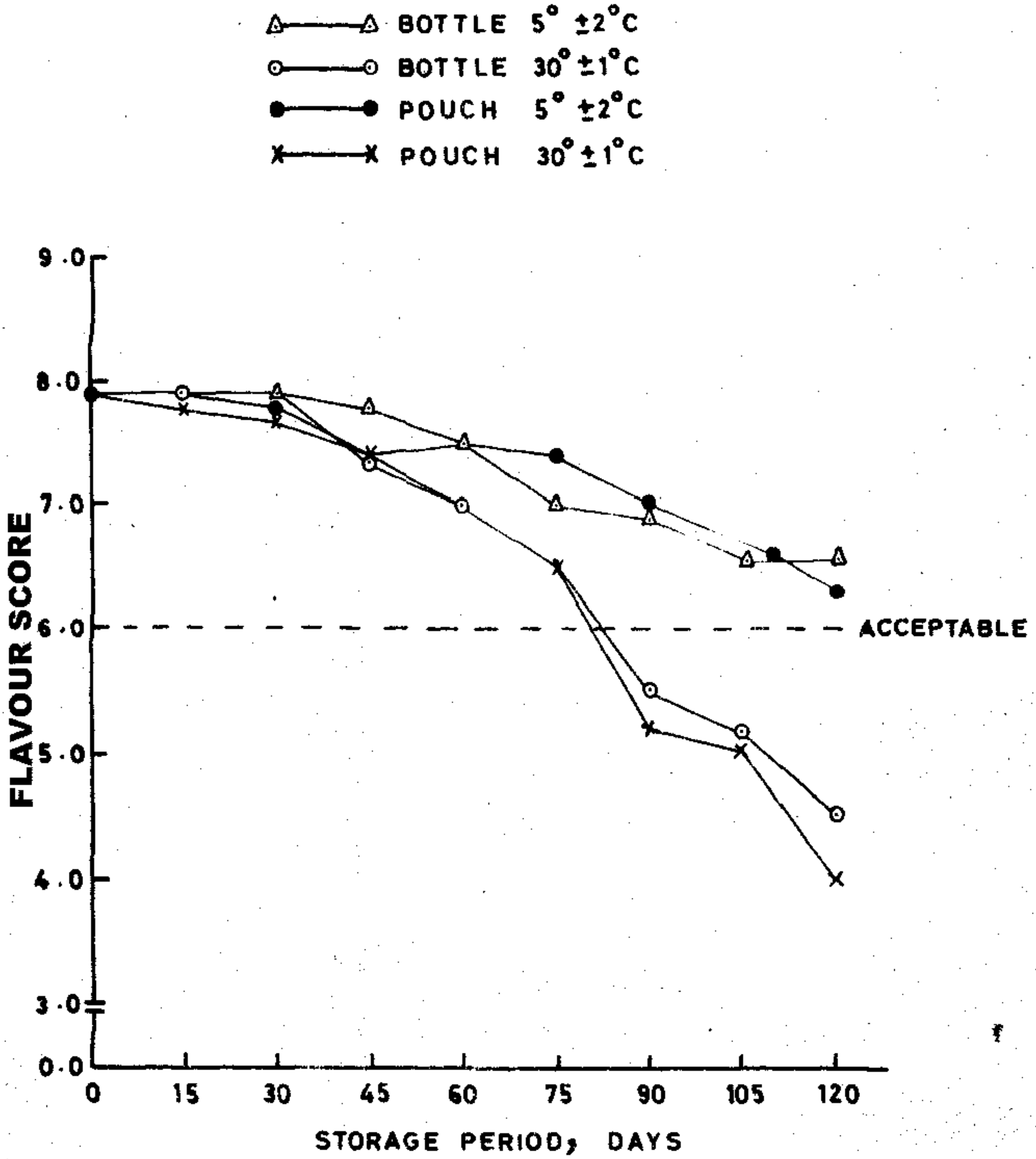

FIG. 5.11. CHANGE IN FLAVOUR SCORE OF ACIDIFIED MILK BEVERAGE DURING STORAGE

were evaluated on a 9-point hedonic scale. On this scale, an average of 6.0 is considered to be slightly acceptable. On the basis of sensory rating, it was found that the flavour of AMB stored at $5^{o} \pm 2^{o}C$ was acceptable upto 120 days of storage in both types of packages. However, at $30^{o} \pm 1^{o}C$, the product had acceptable flavour only upto 75 days (Fig. 5.11).

5.2.4.2 <u>Body and Texture Score</u>

The average body and texture score for freshly prepared AMB was observed to be 7.70 on 9-point hedonic scale. The scores were found to decrease with period of storage. The rate of decrease was faster in samples stored at $30^{o}C$ than at $5^{o}C$. In case of samples stored at $5^{o}C$, the average scores declined to 7.53 and 7.50 for BR and PR, respectively after 120 days of storage. In case of BC and PC, the scores reduced to 7.00 and 6.90 respectively for the same period of storage (Table 5.44). Analysis of variance (Table 5.45) had confirmed that period of storage and temperature of storage significantly ($P < 0.01$) affected the body and texture scores while type of packages had no significant effect. The interaction effect had also shown that only period of storage and temperature of storage (P x T) had significant effect on body and texture score. It is evident from Fig. 5.12, that the rate of decrease in body and texture score in BC and PC was more between 60-90 days of storage period. The lowest average score (6.90)

Table 5.44: Change in body and texture score of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	7.70	7.70	7.70	7.70	7.70
15	7.70	7.70	7.70	7.70	7.70
30	7.70	7.60	7.70	7.57	7.64
45	7.67	7.53	7.70	7.47	7.59
60	7.70	7.37	7.67	7.30	7.51
75	7.63	7.23	7.57	7.03	7.37
90	7.53	7.10	7.67	7.07	7.34
105	7.50	7.10	7.50	7.10	7.30
120	7.53	7.00	7.50	6.90	7.23
Mean	7.63	7.37	7.63	7.31	

Table 5.45: ANOVA for change in body and texture score.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.010	0.71	
Storage periods (P)	8	0.391	28.72**	0.096
Packages (Pk)	1	0.018	1.31	0.045
Temperatures (T)	1	2.253	165.29**	0.045
(P x Pk)	8	0.008	0.60	0.136
(P x T)	8	0.144	10.58**	0.136
(Pk x T)	1	0.024	1.78	0.064
(P x Pk x T)	8	0.003	0.21	0.193
Error	70	0.014		

** $P < 0.01$

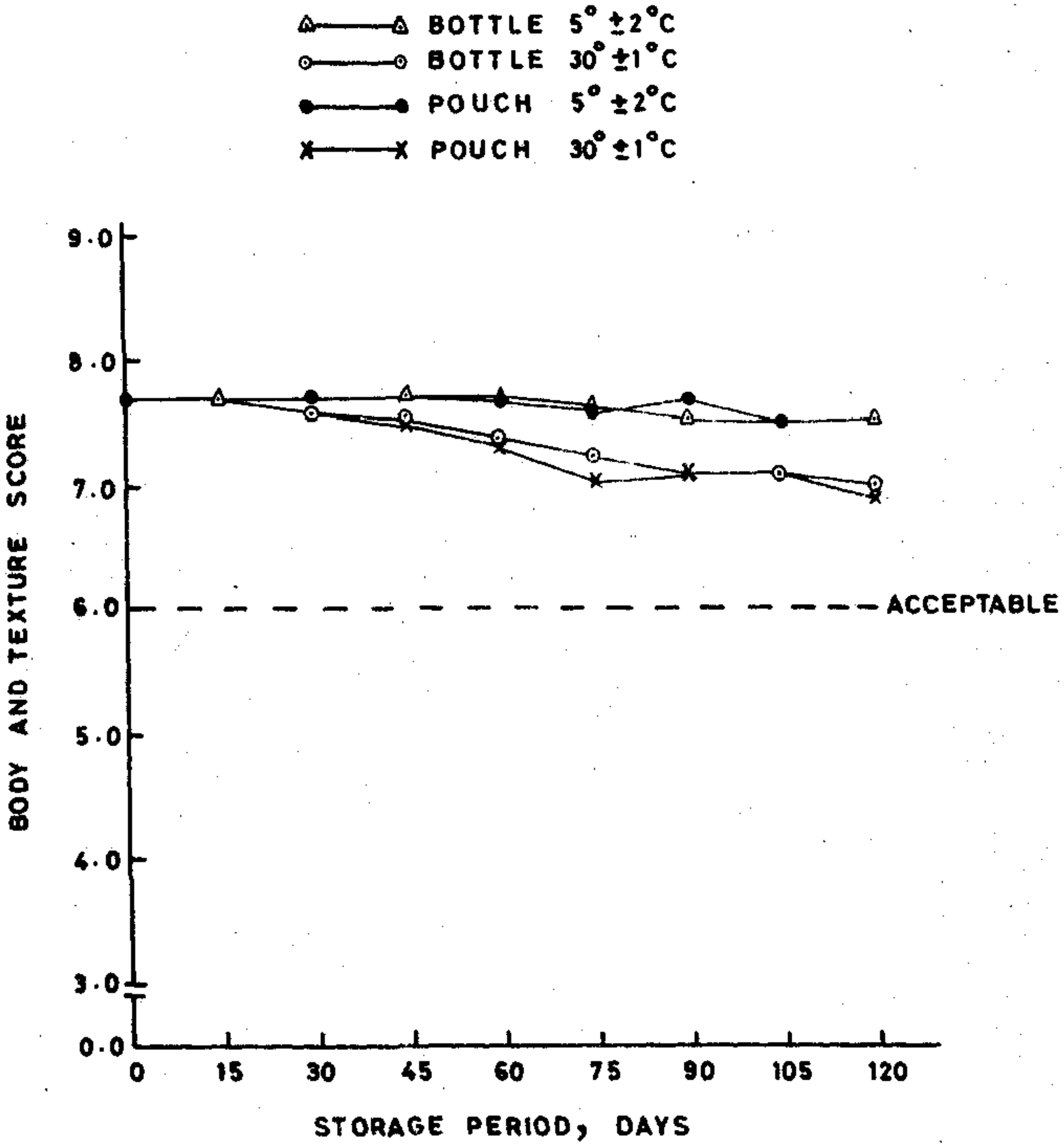

FIG. 5.12. CHANGE IN BODY AND TEXTURE SCORE OF ACIDIFIED MILK BEVERAGE DURING STORAGE

obtained for BC at 30°C, was still higher than the minimum acceptable level (6.0) in the 9-point hedonic scale. This storage study had shown that body and texture of acidified milk beverage was still acceptable after 120 days of storage in samples packaged in two types of packaging materials and stored at two different temperatures.

5.2.4.3 Colour and Appearance Score

Table 5.46 showed that the average colour and appearance score for AMB was 8.0 initially. The score decreased to 7.93, 7.80, 7.90 and 7.70 after 120 days of storage in case of BR, BC, PR and PC, respectively. The analysis of variance (Table 5.47) indicated that the period of storage and temperature of storage had significant effect ($P < 0.01$) on colour and appearance on AMB while, the types of packaging material had no significant effect. The colour and appearance was well above the average acceptable score of 6.0 on 9-point hedonic scale (Fig. 5.13, and Table 5.46).

5.2.4.4 Overall Acceptability Score

It is evident from the Table 5.48 that the initial average overall acceptability score for AMB was 7.80. After 120 days of storage, the average overall acceptability scores ~~were~~ declined to 6.10, 4.47, 6.33 and 4.80 for BR, BC, PR and PC, respectively. From Fig. 5.14, it is evident that the rate of decrease in overall acceptability score was gradual till 30 days of storage. Further storage period caused the scores

Table 5.46: Change in colour and appearance score of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	8.00	8.00	8.00	8.00	8.00
15	8.00	7.97	8.00	7.93	7.97
30	7.90	7.97	8.00	7.97	7.96
45	7.93	7.80	7.97	7.87	7.89
60	7.93	7.90	7.97	7.87	7.92
75	7.90	7.93	7.93	7.90	7.92
90	7.93	7.87	7.93	7.93	7.92
105	7.93	7.93	7.90	7.90	7.92
120	7.93	7.80	7.90	7.70	7.83
Mean	7.94	7.91	7.96	7.90	

Table 5.47: ANOVA for change in colour and appearance score.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.010	1.46	
Storage periods (P)	8	0.029	4.27**	0.068
Packages (Pk)	1	0.000	0.021	0.032
Temperatures (T)	1	0.057	8.56**	0.032
(P x Pk)	8	0.005	0.68	0.096
(P x T)	8	0.012	1.72	0.096
(Pk x T)	1	0.006	0.90	0.045
(P x Pk x T)	8	0.002	0.31	0.136
Error	70	0.007		

** $P < 0.01$

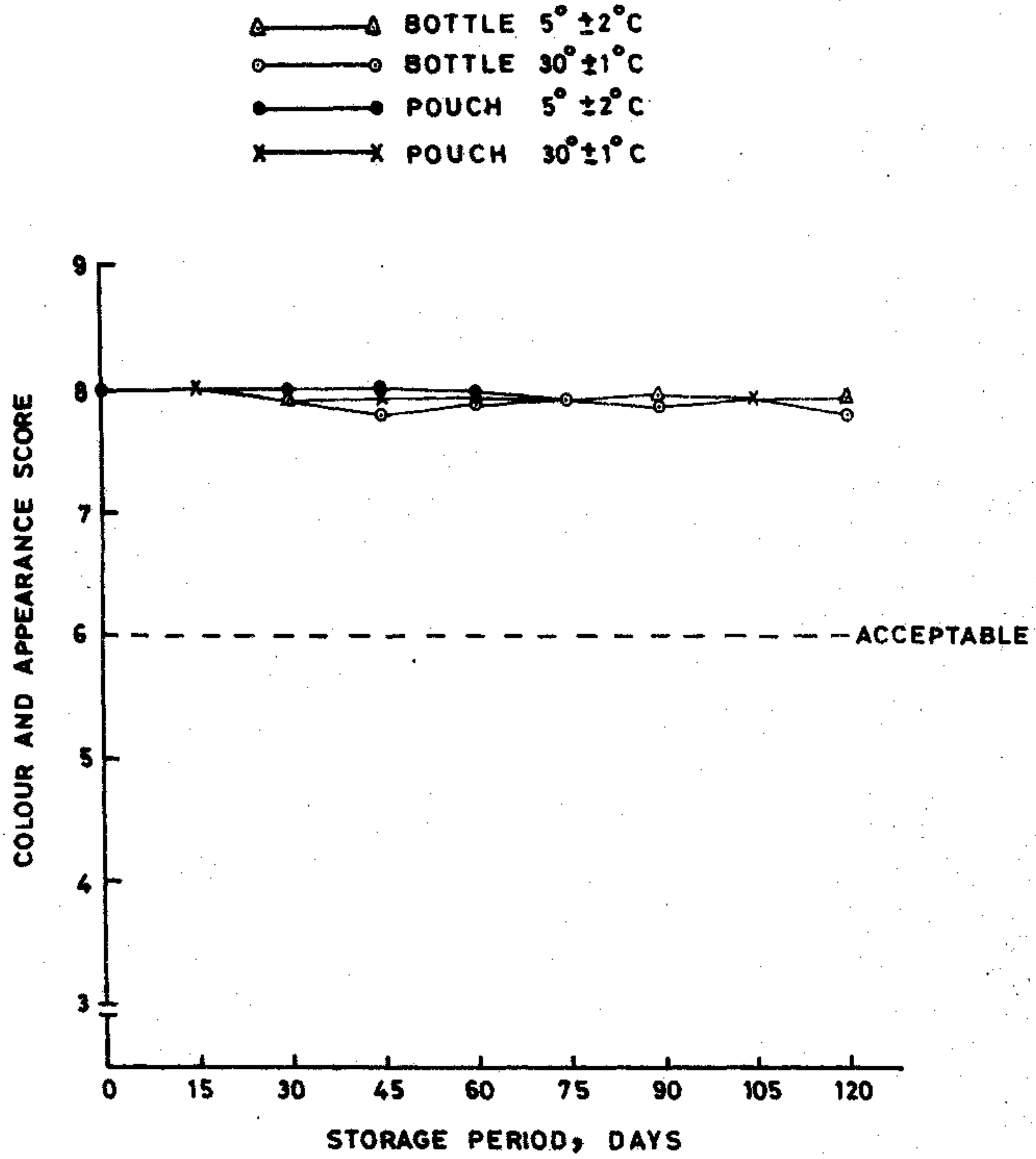

FIG. 5.13. CHANGE IN COLOUR AND APPEARANCE SCORE OF ACIDIFIED MILK BEVERAGE DURING STORAGE

Table 5.48: Change in overall acceptability score of AMB during storage.

Storage period (days)	BR	BC	PR	PC	Mean
0	7.80	7.80	7.80	7.80	7.80
15	7.80	7.80	7.80	7.80	7.80
30	7.73	7.70	7.70	7.70	7.71
45	7.57	7.30	7.70	7.47	7.51
60	7.27	6.90	7.53	6.97	7.17
75	7.00	6.03	7.00	6.30	6.58
90	6.60	5.03	6.60	5.30	5.88
105	6.20	5.00	6.50	5.00	5.67
120	6.10	4.47	6.33	4.80	5.42
Mean	7.12	6.45	7.22	6.57	

to decrease sharply, the decrease being more in samples stored at 30^{o}C. ANOVA (Table 5.49) had also shown that the period of storage, type of package and storage temperature had significant effect ($P < 0.01$) on overall acceptability score of AMB. Among the interaction phenomenon, only period of storage and temperature of storage (P x T) had significant effect ($P < 0.01$). On the basis of average acceptability score (6.0), it is seen that (Fig. 5.14) the product BR and PR are accepta-ble even after 120 days of storage, whereas BC and PC are acceptable upto 75 days of storage.

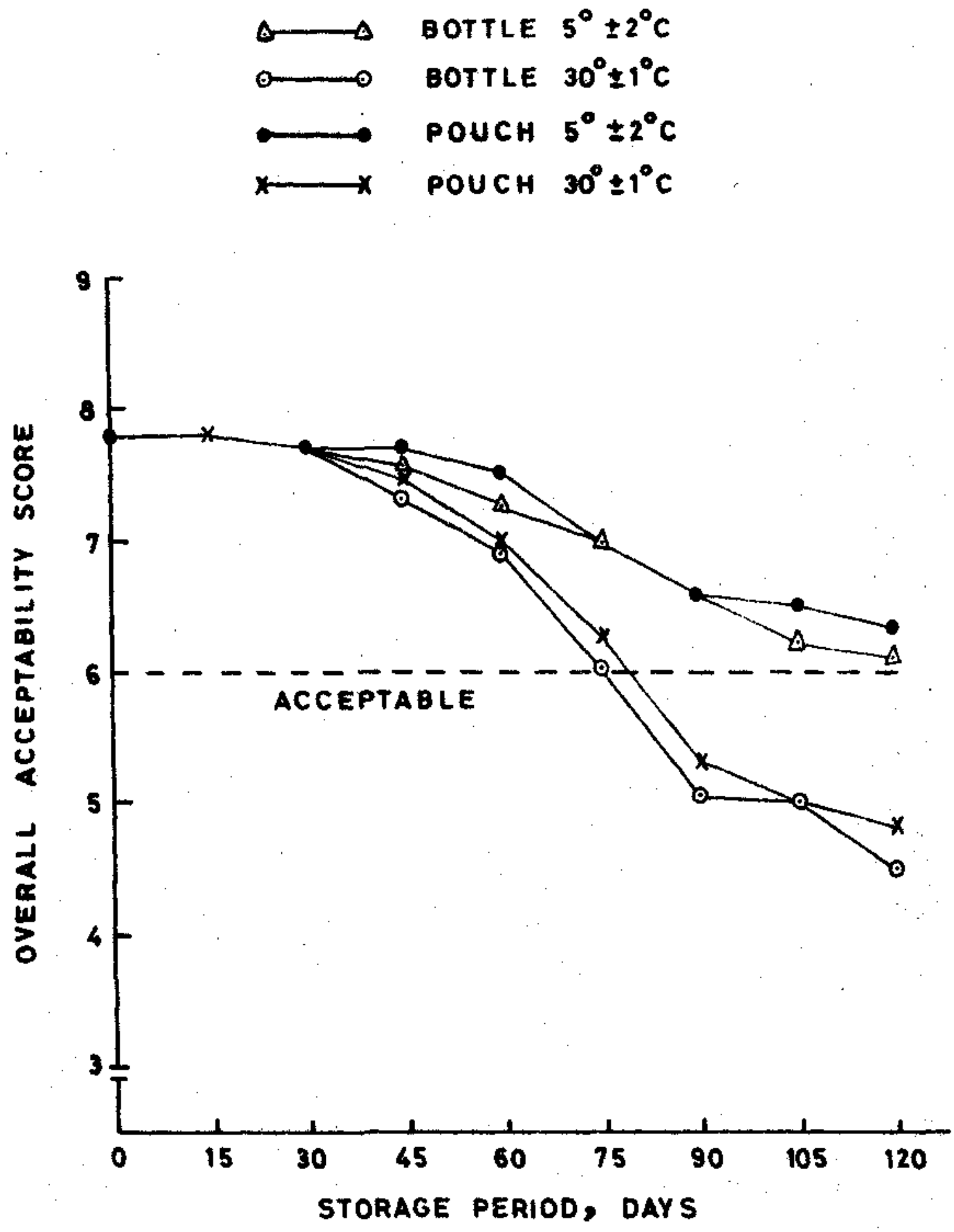

FIG. 5.14. CHANGE IN OVERALL ACCEPTABILITY SCORE OF ACIDIFIED MILK BEVERAGE DURING STORAGE

Table 5.49: ANOVA for change in overall acceptability score.

Source of variation	d.f.	M.S.S.	F.Value	C.D.
Replicates	2	0.005	0.24	
Storage periods (P)	8	11.237	499.10**	0.123
Packages (Pk)	1	0.332	14.75**	0.058
Temperatures (T)	1	11.734	521.18**	0.058
(P x Pk)	8	0.029	1.30	0.175
(P x T)	8	1.293	57.43**	0.175
(Pk x T)	1	0.006	0.25	0.082
(P x Pk x T)	8	0.026	1.15	0.247
Error	70	0.023		

** $P < 0.01$

The sensory characteristics of acidified milk beverage deteriorated during storage. Changes in flavour, colour and appearance, and textural characteristics are important in determining the quality of product during storage. The available results indicated that body and texture characteristics as well as colour and appearance of the product remained acceptable even after 120 days of storage period. But the product was found not acceptable on the basis of flavour scores beyond 75 days of storage when stored at 30°C. However, the product was still acceptable on 120 days when stored at 5°C.

5.2.5 INTER-RELATIONSHIP BETWEEN VARIOUS PHYSICO-CHEMICAL PARAMETERS AND SENSORY ATTRIBUTES

Attempts were made to correlate the changes observed in some physico-chemical properties of acidified milk beverage with that of changes noticed in sensory scores during storage. The coefficients of correlation have been presented in Table 5.50. It could be observed that the sensory scores were found to be highly correlated with physico-chemical parameters of AMB. Based on the information, prediction equations were developed for sensory scores.

5.2.5.1 Relationship between Flavour Score and Physico-chemical Parameters

a) Effect of free fatty acids on flavour:

A highly significant ($P < 0.01$) negative correlation (-0.9411) was observed between flavour scores (FLS) and free fatty acids (FFA). As the FFA content in AMB increased during storage, the sensory score decreased. The relationship between the flavour score and free fatty acids content during 120 days of storage of AMB could be presented by the following equation.

$$FLS = 10.0540 - 2.2262\ FFA \quad \ldots\ldots\ldots \quad (i)$$

Increase in free fatty acids expressed as per cent FFA or acid degree value (ADV) have been reported by many workers in stored UHT treated aseptically packaged

Table 5.50 Coefficients of correlations* among various physico-chemical and sensory parameters of AMB during storage.

PARAMETERS		SED	VIS	RFL	pH	FFA	TBA	NPN	RDS	HMF	FLS	BTS	CAS	OAS
1. Sediment	SED	1.00												
2. Viscosity	VIS	0.81	1.00											
3. Reflectance	RFL	0.73	0.81	1.00										
4. pH	pH	-0.68	-0.82	-0.64	1.00									
5. Free fatty acids	FFA	0.82	0.94	0.80	-0.87	1.00								
6. Thio-barbituric acid value	TBA	0.77	0.91	0.76	-0.86	0.94	1.00							
7. Non-protein nitrogen	NPN	0.77	0.89	0.76	-0.86	0.92	0.94	1.00						
8. Reducing sugar	RDS	0.76	0.88	0.74	-0.86	0.92	0.94	0.98	1.00					
9. Hydroxy Methyl Furfural value	HMF	0.74	0.87	0.73	-0.82	0.90	0.92	0.98	0.98	1.00				
10. Flavour score	FLS	-0.82	-0.89	-0.79	0.82	-0.95	-0.92	-0.90	-0.90	-0.87	1.00			
11. Body & Texture score	BTS	-0.73	-0.78	-0.71	0.72	-0.81	-0.82	-0.89	-0.87	-0.87	0.86	1.00		
12. Colour & appearance score	CAS	-0.48	-0.46	-0.43	0.39	-0.44	-0.38	-0.47	-0.48	-0.45	0.44	0.39	1.00	
13. Overall acceptability score	OAS	-0.82	-0.93	-0.79	0.84	-0.96	-0.93	-0.91	-0.89	-0.88	0.94	0.84	0.40	1.00

* Values give linear correlation coefficients

milk (Schmidt and Renner, 1978; Wadsworth and Bassette, 1985). Singh and Patil (1989) have reported a consistent rise of ADV in UHT buffalo milk (both in 5% and 6% fat) during storage. Renner (1979) pointed out that the release of FFA during storage of UHT milk is a result of fat hydrolysis by exocellular lipases of Pseudomonas species which are not inactivated during UHT processing. No such information is available on stored directly acidified milk beverage.

b) Effect of thio-barbituric acid on flavour:

The relationship between the deterioration in flavour score (FLS) and thio-barbituric acid (TBA) values during four months of storage period could be given by the following equation.

$$FLS = 8.4722 - 39.4999\ TBA \quad \ldots\ldots\ldots \quad (ii)$$

Correlation coefficient obtained for AMB was -0.9210, which showed highly significant negative relationship ($P < 0.01$) between flavour deterioration and increase in TBA values of beverage samples.

Dunkley and Jennings (1951) and Aurand et al. (1959) had also established a clear relationship between the TBA values and the development of an oxidized flavour. Mottar et al. (1979) while studying sensory changes in UHT milk stored uncooled (20°C) upto 4 months duration, observed that despite deterioration in taste, TBA values did not exceed noticeable level (above 0.055) and hence he disclaimed a possible contribution of fat oxidation to deterioration in taste.

In case of AMB, faster deterioration in taste was observed after 75 days of storage at 30°C (Fig. 5.3). The reason for faster deterioration of sterilized AMB (prepared from buffalo milk) could be attributed to its higher degree of unsaturated fatty acids content, lower concentration of α-tocopherol and other inherent properties which make buffalo milk fat more prone to oxidative deterioration (Rama Murthy and Narayanan, 1974).

c) Effect of non-protein nitrogen on flavour:

A highly significant ($P < 0.01$) relationship was obtained ($r = -0.9044$) between non-protein nitrogen (NPN) content and flavour scores (FLS) during storage, that was expressed by the equation.

$$FLS = 10.9710 - 11.0760\, NPN \quad \ldots\ldots \quad (iii)$$

UHT milk samples that developed bitter taste before gelation occurred, exhibited increase in NPN content from 0.03 to 0.06 per cent, but there was no common level of NPN before which the level of bitterness could be considered as unacceptable (Mitchell and Ewings, 1985). However, in present studies the maximum increase in NPN content at 30°C storage temperature was observed to be 0.0595 per cent (Table 5.28). Even at this level of NPN, no bitter taste was observed in the product.

d) Effect of hydroxy-methyl furfural value on flavour:

The relationship between the flavour scores (FLS) and total HMF contents during 120 days storage of AMB was expressed by the equation.

$$FLS = 8.5201 - 0.1871\ HMF \ldots\ldots\ldots \quad (iv)$$

A negative correlation ($r = -0.8744$) was obtained. It was reported that high HMF concentration (above 5 ppm) is related with stale flavour and bitter taste development in stored UHT milk (Mottar et al., 1979). In AMB during storage period of four months at controlled temperature (30°C) the total HMF content was observed to be maximum 23.15 μ mol/lit which could have affected the flavour scores during storage period.

e) Effect of reducing sugar and pH on flavour:

The correlation between flavour scores and changes noticed in reducing sugar (RDS) and pH was not very high as compaired to FFA and TBA value. The regression of flavour (FLS) on reducing sugar and pH being expressed as:

$$FLS = 10.2743 - 0.7717\ RDS \ldots\ldots\ldots\ldots \quad (v)$$

and $$FLS = -153.1359 + 42.8504\ pH \ldots\ldots\ldots \quad (vi)$$

The coefficients of determination (R^2) for reducing sugar and pH were 0.8125 and 0.6676, respectively. It may be explained that about 81.55 per cent and 66.76 per cent of changes in flavour during storage would be due to change in reducing sugar and pH, respectively.

f) Relative contribution of intrinsic parameters to flavour changes:

The relative contribution of intrinsic parameters, such as FFA, TBA value and NPN content to the flavour scores of AMB during storage was studied. The following multiple linear regression equations were obtained:

$$FLS = 9.6886 - 1.6648\ FFA - 10.8310\ TBA \quad \dots\dots (vii)$$

$$FLS = 10.0535 - 1.5450\ FFA - 6.6290\ TBA - 1.9109\ NPN \quad \dots\dots (viii)$$

The correlation coefficient (r) and coefficients of determination (R^2) for (vii) and (viii) multiple parameters were, $r = -0.9497$, $R^2 = 0.9019$; and $r = -0.9511$, $R^2 = 0.9047$, respectively. It was, therefore concluded that 90.19 per cent and 90.47 per cent of the flavour changes during storage of AMB were explained by these intrinsic parameters.

5.2.5.2 <u>Relationship between Body and texture score and Physico-chemical parameters:</u>

An interdependance of body and texture scores (BTS) with those of viscosity (VIS) and sediment (SED) of the product was observed and regression of the former on the latter parameters could predict 61.75 per cent and 53.11 per cent variation, respectively in the body and texture score. The individual relationship of viscosity and sediment content with body and texture scores have been presented in the following equation.

$$BTS = 11.3952 - 0.4239\ VIS \quad (r = -0.7658) \quad \ldots\ldots\ldots\ldots \text{(ix)}$$

$$BTS = 8.0177 - 1.6492\ SED \quad (r = -0.7287) \quad \ldots\ldots\ldots\ldots \text{(x)}$$

The predictability of body and texture was improved when its regression was taken on viscosity and sediment together ($R^2 = 0.6433$)

$$BTS = 10.5027 - 0.3056\ VIS - 0.6154\ SED \quad (r = -0.8021) \quad \ldots\ldots \text{(xi)}$$

5.2.5.3 Relationship between Colour and Appearance and Physico-chemical parameters:

a) Effect of HMF and reflectance on colour and appearance score:

Average colour scores for sterilized acidified milk beverage remained almost consistent during complete storage period (Fig. 5.13) and no apparent change in colour was noticeable. Change in colour was expected mainly due to Maillard reaction between the lysine and lactose in sterilized fluid milk products.

Attempts were made to find out correlation between colour and appearance scores (CAS) and total HMF content and reflectance values (RFL).

The colour and appearance scores were found to be not significantly correlated with HMF (r = -0.4481), with reflectance (r = -0.4317) and also reflectance along with HMF (r = -0.4736).

A non-significant relationship between HMF content and CAS showed that the HMF concentration had to be very high to obtain perceivable change in colour, since the concentration of HMF is an indirect measure of extent of browning reactions that had occurred in milk (Burton, 1956; Swartzel _et al._, 1980). Further, it should be noted that a poor correlation presumably due to the fact that HMF is only an intermediate product in the complex reactions involved in Maillard browning leading to formation of brown melanoidins as the end product or it is possible that added colour in the product interfered with the apparent change in colour of the product.

b) Effect of HMF on reflectance:

The predictability of colour in terms of reflectance (RFL) was appreciably improved when correlated with total HMF values. The regression equation obtained is given as under.

$$RFL = -0.4117 + 0.2451\ HMF \quad \ldots\ldots\ldots \quad (xii)$$
$$R^2 = 0.5317$$

The reflectance values and total HMF values were significantly correlated ($r = +0.7292$; $P < 0.01$) and could predict 53.17 per cent change in reflectance due to a unit change (0.2451) in HMF values during storage.

522.5.4 Relationship between Overall Acceptability Score and Physico-chemical Parameters

The overall acceptability is a combined effect of all sensory attributes. In finding correlation between flavour scores, maximum correlation was obtained in case of changes noticed in FFA content. Similarly, among body and texture scores, viscosity was found to have higher correlation with body and texture scores. In case of colour and appearance score, a non-significant relationship was observed with some of the factors like changes in HMF and reflectance. But it was found that colour and appearance in terms of reflectance could be possible to predict with change in HMF values. Considering most relevant relationship among sensory attributes and physico-chemical parameters, an attempt was made to find out few suitable regression equations, which could become more helpful to predict overall acceptability (OAS) of AMB during storage. The following linear equations were obtained:

$$OAS = 0.6268 + 0.8938\ FLS \quad \ldots\ldots\ldots \quad (xiii)$$
$$R^2 = 0.8938; \quad r = +0.9454$$

$$OAS = 13.4861 - 1.6738\ FFA - 0.4678\ VIS \quad \ldots \quad (xiv)$$
$$R^2 = 0.9348; \quad r = -0.9668$$

$$OAS = 13.0970 - 1.6427\ FFA - 0.4259\ VIS - 0.0249\ RFL$$
$$R^2 = 0.9354; \quad r = -0.9671 \quad \ldots\ldots\ldots \quad (xv)$$

$$OAS = 12.3665 - 1.2460\ FFA - 7.4860\ TBA - 0.7462\ SED - 0.3538\ VIS \quad \ldots \quad (xvi)$$
$$R^2 = 0.9405; \quad r = -0.9698$$

Among sensory attributes, flavour scores (FLS) of the product being contributed significant, ($r = +0.9454$; $P < 0.01$) to the overall acceptability score (OAS). Among relative contribution of intrinsic parameters, FFA content and viscosity of the product together contributed 93.48 per cent to OAS as compared to other physico-chemical parameters and hence equation (xiv) can suitably be used for predicting the shelf life of the directly acidified milk beverage during storage.

5.3 COST ESTIMATION OF ACIDIFIED MILK BEVERAGE

The cost of production is an important consideration for establishing a remunerative project and successful marketing of the product. The cost of any product depends on a number of variable factors and capital facilities available. Full utilization of capacity, services, buildings, equipment, and manpower and administrative set-up generally reduce the cost of production of the food product.

In the present study, the cost of production of acidified milk beverage was calculated under three heads viz., cost of raw materials, cost of processing and cost of packaging on the basis of assumptions given in section 4.9.

5.3.1 COST OF RAW MATERIALS

The beverage factory with the capacity to handle 1000 kg of milk (6.0% fat and 9.0% SNF) per day is expected to product 4,97,490 litres of acidified milk beverage per year (from 1000 kg of milk, 1658.3 litres of beverage is expected after assuming 1.0 per cent loss during manufacture). The raw materials' cost included expenditure on ingredient inputs used in the manufacture of the beverage and were calculated (Table 5.51) to be Rs. 1.75 per 200 ml of acidified beverage.

Table 5.51: Cost of raw materials.

Ingredients	Quantity per annum	Rate per kg or lit, Rs.	Amount (Rs)
Milk	3,00,000 kg	5.50	16,50,000
Sugar	76,654 kg	8.50	6,51,559
Stabilizer	3,428 kg	675.00	23,13,900
Acid	3,994 kg	25.00	99,850
Stabilizing salt	383 kg	25.00	9,575
Colour	12.6 kg	130.00	1,638
Flavour	503 lit	140.00	70,420
		Total	47,96,942
Return from sale of cream @ Rs.25.00/kg (74.5 kg of 40% cream per day)			- 5,58,750
Cost of raw materials per annum			42,38,192
Interest on working capital @ 16.5% per annum for two months			1,16,550
		Total:	43,54,742

5.3.2 COST OF PROCESSING

For the purpose of computing the cost of processing (Table 5.57) the investment on labour, power and utilities (steam, electricity, water, etc), operating supplies, laboratory charges, refrigeration, depreciation etc. were considered as detailed below.

5.3.2.1 Skilled and Unskilled Labour Charges

In order to manufacture 407490 litres of AMB per year, the type and number of personnel needed and their wages (as per current regulations) have been detailed in Table 5.52.

Table 5.52: Expenditure by way of labour charges.

Personnel	Numbers	Salary/person per month (Rs)	Total exp. per annum
1. Factory manager	1	2500	30,000
2. Shift incharge	1	2000	24,000
3. Technicians	4	1500	72,000
4. Semi-skilled worker	5	1000	60,000
5. Unskilled labour	10	800	96,000
6. Clerk (office-cum-store)	1	1500	18,000
		Total:	3,00,000

5.3.2.2 Power and Utilities

The expenditure on the power and utilities was calculated on the basis of individual component consumption during the period of the beverage manufacture.

a) Electricity charges:

The expenditure on electricity consumption was opportioned on the basis of horse power hours (HPH) taken as equal to 1 KWH electricity. Horse power hours were calculated by multiplying the horse power of a motor with the number of hours if worked during the year for a particular operation (Table 5.53). The electricity is to be purchased in bulk and the cost of 1 kwh (per unit) is taken as Rs. 1.00.

Table 5.53: Electricity charges

Name of unit	No.	HP of motor	Hours used per annum	HPH per annum	Cost per annum (Rs)
1. Milk pump	2	1.0	600	1200	
2. Cream separator	1	0.75	150	112.5	
3. Rotary sterilizer	1	10.00	1500	15000	32138.00
4. Homogenizer	1	7.50	510	3825	
5. Refrigeration unit	1	5.00	2400	12000	
				32137.5	
			or	32138.0	

b) Steam charges:

It was assumed that the steam generated by mini boiler (capacity: 50 kg of steam per hour) would be sufficient for different processes, such as fore-working, heating, sterilization, cleaning etc. The total expenditure on steam in the form of electricity charges have been calculated as follows:

Electricity consumption rate ---- 20 kwh/hour
Hours used per annum @ 2.50 hrs/day ---- 750 hours

Therefore, kwh per annum @ 20 kwh/hour would be
...... 15,000 kwh;

Therefore, cost per annum @ Rs. 1.0/kwh would be
...... Rs. 15,000

c) Water charges:

The amount of water required for processing as well as cleaning during the manufacture of acidified milk beverage was considered to cost about Rs. 20.00 per day. Therefore, total expenditure on water per annum would be Rs. 6000.00

Table 5.54: Expenditure on Power and Utilities.

Component		Amount per annum (Rs)
1. Electricity		32,138
2. Steam		15,000
3. Water		6,000
	Total	53,138

5.3.2.3 Charges for Operating Supplies

The expenditure incurred by various consumable articles required for proper and smooth functioning of the factory, have been detailed as follows.

Table 5.55: Charges for operating supplies.

Supplies	Cost per annum (Rs)
1. Cleaning and sanitizing materials	
Teepol	2,500
Sodium hydroxide	2,000
Washing soda	2,000
Nitric acid	1,700
Bleaching powder	1,800
2. Laboratory charges (Glasswares and reagents)	7,500
3. Spare and maintenance of equipments and machineries	15,000
4. Miscellaneous	12,000
Total	44,500

5.3.2.4 Depreciation of Building and Equipments

For calculating depreciation (Table 5.56) on building and equipment, a 'useful life' and 'salvage value' at the end of useful life were assumed. The annual cost due to depreciation was calculated as under:

$$\text{Annual cost due to depreciation} = \frac{\text{Initial cost - salvage value}}{\text{Years of useful life}}$$

Table 5.56: Depreciation on building and equipments.

Building and equipments	Original value (Rs)	Salvage value (Rs)	Depreciation (%)	Depreciation per annum (Rs)
Building	5,00,000	5,000	2.5	12,375
Weighing scale	5,000	250	5.0	237.50
Milk receiving tank	50,000	2500	5.0	2,375
Multipurpose tank with stirrer	1,00,000	5000	5.0	4,750
Milk standardization tank	10,000	500	5.0	475
Milk pumps	14,000	700	5.0	665
Cream separator	15,000	750	5.0	712.50
Homogenizer	2,70,000	8100	5.0	13,095
Mini boiler	30,000	1500	5.0	1,425
Batch type sterilizer	25,000	1250	5.0	1187.50
Aluminium milk cans	30,000	1500	5.0	1,425
Can washing unit (manual)	5,000	250	5.0	237.50
Can steaming block	2,000	100	5.0	95
Bottle washing machine (hand operated)	5,000	250	5.0	237.50
Bottle filling machine (hand operated)	5,000	250	5.0	237.50
Crowncorking machine (hand operated)	2,500	125	5.0	118.75
Refrigeration unit for cold storage	60,000	1800	5.0	2910.00
Total:	11,28,500	29825		42558.75

5.3.2.5 Interest on Building and Equipments

The interest at the rate of 16.5 per cent per annum was calculated on total investment of Rs. 11,28,500, was found to Rs. 1,86,202.50 per year.

5.3.2.6 Total cost of Processing

The total cost of processing for 497490 lit. of acidified milk beverage per annum would be Rs. 6,26,399 as per the details given in Table 5.57. The cost of processing per bottle (200 ml beverage) worked out to be Rs. 0.2518 or 0.25.

Table 5.57: Total expenses incurred in processing.

	Component	Amount (Rs)
1.	Skilled and unskilled labour	3,00,000
2.	Power and utilities	53,138
3.	Operating supplies	44,500
4.	Depreciation on building and equipments	42,558.75
5.	Interest on building and equipments	1,86,202.50
	Total	6,26,399.25 or 6,26,399.00

5.3.3 COST OF PACKAGING

The cost of packaging materials including glass bottles, crown corks and wooden crates were considered as consumable items. The cost due to these items were calculated as per assumptions given in Table 5.58.

Table 5.58: Cost of packaging.

Items	Assumptions	Cost per annum (Rs)
1. Glass bottles :	Cap. 200 ml Rate: Rs. 2.50/bottle Stock: 12 days Consumption: In 25 trips Interest @ 16.5% per annum for 12 months	2,89,788
2. Crown corks	Rate : Rs.0.12/cork Stock : 1 month Consumption: Single service Interest @ 16.5% per annum for one month	3,02,598
3. Wooden crates :	Cap : 24 bottles/crate Rate : Rs. 25.0/crate Stock : 12 days Consumption: In 100 trips Interest @ 16.5% per annum for 12 months	42,952
	Total:	6,35,338

5.3.4 TOTAL COST OF MANUFACTURE OF ACIDIFIED MILK BEVERAGE

The break-up of the total cost of manufacture per bottle (200 ml beverage) of acidified milk beverage has been presented in Table 5.59.

Table 5.59: Estimated cost of manufacture of acidified milk beverage.

Component	Cost per annum (Rs)	Cost per bottle (Rs)
1. Raw materials	43,54,742	1.7507 or 1.75
2. Processing	6,26,399	0.2518 or 0.25
3. Packaging	6,35,338	0.2554 or 0.26
Total	56,16,479	2.2579 or 2.26

The cost of raw materials, processing, packaging and total cost of manufacture, for per bottle (200 ml) of ready beverage was found to be 1.75, 0.25, 0.26 and 2.26 rupees, respectively. If the volume of operation is increased beyond 1000 kg per day, the cost of manufacture per bottle is expected to come down further.

CHAPTER - 6

SUMMARY AND CONCLUSION

6.0 SUMMARY AND CONCLUSION

6.1 Direct acidification is a new approach in making products similar to culture type dairy products. Attention is drawn to the growing awareness in developing novel type milk based beverages and to the scope for development of directly acidified milk based beverage. Emphasis is also laid on the importance of appropriate packaging system especially the aseptic packaging.

6.2 Manufacturing techniques attempted in the preparation of various types of acidified milk beverage have been reviewed. Effect of addition of hydrocolloids in improving the protein stability in acidified milk system and their role in certain dairy product manufacture have been discussed.

6.3 The scope of the present investigation included standardisation techniques for the manufacture of acidified milk beverage, study of physico-chemical, microbiological and storage characteristics of AMB and computation of cost of production of acidified milk beverage as per the standardised technique.

6.4 Materials and methods used in the preparation of acidified milk beverage and the various analytical techniques used in evaluating the product are elaborated.

6.5 Process optimisation was carried out for the manufacture of acidified milk beverage.

6.5.1 Selection of pH-sugar combination and type of acid was attempted.

6.5.1.1 Preliminary laboratory trials on selection of pH-sugar combination with different types of acids indicated that acidified beverage samples prepared using phosphoric acid were more acceptable at pH 3.75 with four levels (14,16,18 and 20%) of sugar. With lactic acid, a pH of 4.25 was found to be more acceptable. In case of citric acid AND prepared with a pH of 4.0 and 4.25 was found to be more acceptable. In case of use of hydrochloric acid none of the samples were found acceptable during preliminary trials itself.

6.5.1.1.1 Studies on optimisation of pH and sugar level using citric acid had shown that beverage prepared with pH of 4.0 and 18 per cent sugar was most acceptable.

6.5.1.1.2 Optimisation of pH and sugar level using phosphoric acid had shown that a combination of pH 3.75 and 18 per cent sugar level was most acceptable.

6.5.1.1.3 Studies on optimisation of pH and sugar level using lactic acid had shown that beverage prepared with pH of 4.25 and 16 per cent sugar was most acceptable.

6.5.1.1.4 Based on the flavour scores obtained for acidified milk beverage prepared with citric, phosphoric and lactic acid with four levels of sugar, it was found that maximum flavour score was obtained by sample prepared with phosphoric acid at a pH of 3.75 and with 18 per cent sugar.

6.5.1.2 Stabilization of protein in acidified milk system was attempted with addition of stabilizers.

6.5.1.2.1 Studies on initial selection had indicated that two types of pectin and two grades of CMC could be tried for improving the stability of acidified milk system.

6.5.1.2.2 Screening studies on stabilizers indicated that stability of acidified milk system could be improved as the level of carboxymethyl cellulose (medium viscosity) was increased from 0.8 to 1.2 per cent.

6.5.1.2.3 Studies on optimisation of level of CMC in acidified milk system had shown higher flavour, body and texture score and lower viscosity in AM8 could be obtained when the level of CMC was maintained at 1.15 per cent level in toned milk.

6.5.1.3 Trials on optimisation of level of milk solids in AM8 had indicated that dilution of toned milk with water upto a ratio of 70:30 improved the organoleptic score. Further dilution caused the sensory scores to decline.

6.5.1.4 Among the two stabilizing salts tried in beverage to improve the sensory scores, addition of both had shown improvement in certain sensory scores. The improvement was observed to be more in case of trisodium citrate than diabasic potassium phosphate. Trisodium citrate at 0.09 per cent level gave higher sensory scores.

6.5.1.5 Studies on optimisation of type and level of added flavour in AMB had shown pineapple flavour at the rate of 1.0 ml per litre of beverage was most acceptable.

6.5.1.6 Consumer preference trials on colour level in AMB had shown that lemon yellow colour at the rate of 2.5 ml of 1 per cent solution, per litre of beverage was found to be more acceptable.

6.5.1.7 Based on the optimisation trials, a standardised technique for the manufacture of AMB is suggested. The analysis of AMB had shown the pH of the product varied between 3.74 to 3.76 with an average of 3.75. The average total solids (per cent), fat (per cent) and protein (per cent) were found to be 22.04, 1.69 and 1.79 respectively. The average viscosity of AMB was observed to be 8.701 cP.

6.5.1.8 The consumer acceptance trial on AMB had shown that out of 530 consumer, 103 (19.4%) rated this product as excellent and 211 (39.81%), 176 (33.21%), 34 (6.42%) and 6 (1.13%) rated the product as very good, good, fair and poorly acceptable, respectively. It was also observed that the liking of the product differed with different age groups. Further it was noticed that the acceptability scores were more or less similar among both male and female consumer.

6.5.2 Shelf-life studies on acidified milk beverage carried out for 120 days at $5^{o} \pm 2^{o}C$ and $30^{o} \pm 1^{o}C$ temperature.

6.5.2.1 Shelf life studies on AMB indicated that FFA content increased during the storage period. Maximum increase (2.720 μ eq/ml) in FFA was noticed in BC followed by PC, BR and PR. Rate of increase in FFA content was observed to slow upto 45 days of storage but increased at a faster rate during subsequent period, the rate of increase being far more at 30^{0}C of storage. The rate of increase in FFA content was observed to be more in samples stored in glass bottles than in metallised polyester pouches.

6.5.2.2 The TBA value (expressed as OD) of AMB increased during storage from 0.019 to 0.044, 0.166, 0.044 and 0.093 for samples, BR, BC, PR and PC, respectively. The rate of increase in TBA value was observed to be faster after two months of storage. The increase in TBA value was observed to slightly higher in glass bottles than in metallised polyester pouches.

6.5.2.3 NPN content of AMB was observed to increase from the initial value of 0.0285 per cent to 0.0348, 0.0595, 0.0336 and 0.0565 per cent in BR, BC, PR and PC, respectively. The increase in NPN content was observed to be slightly more in case of samples stored in bottles than in metallised polyester pouches.

6.5.2.4 The reducing sugar level in AMB increased from an initial value of 3.33 per cent to 4.21, 7.89, 3.86 and 7.59 for BR, BC, PR and PC, respectively. The increase in reducing sugar content was observed to be more in samples stored in bottles than in metallised polyester pouches.

6.5.2.5 The total HMF content increased from an initial value of 3.93 μ mol/lit to 7.15, 23.15, 6.50 and 18.57 μ mol/lit in BR, BC, PR and PC respectively. The rate of increase was observed to be more in samples stored in bottles than in metallised polyester pouches.

6.5.2.6 The pH in AMB was observed to decrease significantly after 45 and 60 days of storage in BC and PC respectively, there was no significant change in BR and PR almost upto 90 days.

6.5.2.7 The sediment formation in AMB showed that it increased from initial value of 0.17 ml/10 ml to 0.40, 0.55, 0.40 and 0.58 ml/10 ml in BR, BC, PR and PC, respectively. Though the sediment formation showed increasing trend during storage period, the changes noticed were undulating.

6.5.2.8 Studies on the changes in viscosity of AMB during storage had shown that the values increased from 8.701 cP to 9.610, 10.589, 9.440 and 10.294 cP for BR, BC, PR and PC, respectively. The rate of viscosity increase was observed to be higher at 30^{o}C storage temperature than at 5^{o}C.

6.5.2.9 Change of colour in AMB measured as per cent drop in reflectance was not perceptible upto 30 days of storage in samples stored at 5^{o}C. In case of higher storage temperature of 30°C, no change in reflectance noticed till 15 days of storage. The decrease in per cent reflectance was quite considerable during storage after 30 days.

6.5.2.10 Total spore count of AMB samples were observed to nil upto 60 days of storage. After 120 days of storage the counts were found to be 4, 53, 12 and 44 in BR, BC, PR and PC, respectively, indicating probably the surviving spores germinating due to changes in environmental condition in the product during storage.

6.5.2.11 The flavour scores of AMB decreased during storage from initial value of 7.90 to 6.60, 4.50, 6.30 and 4.00 for BR, BC, PR and PC, respectively, after 120 days of storage. The period and temperature of storage were found to have significant effect on flavour while type of packaging material used had no effect on flavour during storage. On the basis of sensory rating it is observed that the flavour score of AMB stored at 5°C was acceptable upto 120 days of storage. At 30°C of storage, the product had acceptable flavour score only upto 75 days.

6.5.2.12 Body and texture scores were also found to decrease with the period of storage. In case of samples stored at 5°C, the average scores declined to 7.53 to 7.50 from the initial score of 7.70 in BR and PR, respectively, after 120 days. In case of BC and PC the scores were

7.00 and 6.90 respectively for the same period. In all samples the body and texture scores were found to be acceptable even after 120 days of storage.

6.5.2.13 The colour and appearance score for AMB decreased from initial score of 8.0 to 7.93, 7.80, 7.90 and 7.70 for BR, BC, PR and PC respectively, after 120 days of storage. The colour and appearance score were found to be well above the average acceptable score of 6.0 on 9-point hedonic scale during the entire period of storage under present investigation.

6.5.2.14 The overall acceptability score of AMB decreased from the initial score of 7.80 to 6.10, 4.47, 6.33 and 4.80 for BR, BC, PR and PC, respectively, after 120 days of storage. Samples of BR and PR stored at 5°C were found to be acceptable even upto 120 days of storage whereas samples of BC and PC were acceptable upto 75 days of storage on the basis of acceptability of score of 6.0 on 9-point hedonic scale.

6.5.2.15 Attempts were made to correlate the changes observed in some physico-chemical properties of AMB with that of changes noticed in sensory scores.

6.5.2.15.1 A highly significant negative correlation (-0.9461) was observed between flavour score and FFA content in AMB, between TBA value and flavour score (-0.9210), NPN and flavour score (-0.9044) and HMF value and flavour score (-0.8744). The correlation between flavour scores and changes noticed in reducing sugar content and pH of AMB were not high as compared to FFA and TBA. The relative contribution of intrinsic parameters such as FFA, TBA value and NPN content to the flavour scores of AMB during storage had shown that 90.19 per cent and 90.47 per cent of the flavour changes during storage of AMB were explained by these intrinsic parameters.

6.5.2.15.2 The predictability of body and texture score was observed to be improved when its regression was considered on viscosity and sediment together ($R^2 = 0.6433$).

6.5.2.15.3 Colour and appearance scores were not found to be significantly correlated with HMF value ($r = -0.4481$) with reflectance ($r = -0.4317$) and also reflectance along with HMF ($r = -0.4736$). The reflectance values and total HMF values were observed to be significantly correlated ($r = +0.7292$) and could predict 53.17 per cent changes in reflectance due to unit change (0.2451) in HMF values during storage.

.5.2.15.4 Among sensory attributes, flavour scores (FLS) contributed significantly ($r = 0.9454$) to the overall acceptability score (OAS). Among relative contribution of intrinsic parameters, FFA content and viscosity of the product together were found to contribute 93.48 per cent to OAS as compared to other physico-chemical parameters.

6.5.3 The cost of production of acidified milk beverage as per the standardised technique was worked out under three heads (i) cost of raw material, (ii) cost of processing and (iii) cost of packaging.

6.5.3.1 Raw material cost has been worked to be Rs. 1.7507, Rs. 1.75 per 200 ml of acidified beverage.

6.5.3.2 Cost of processing has been worked out to be Rs. 0.2518 or Rs. 0.25 per 200 ml of acidified milk beverage.

6.5.3.3 Cost of packaging is worked out to be Rs. 0.2554 or Rs. 0.26 per 200 ml of acidified milk beverage.

6.5.3.4 The total cost of manufacturing of AMB packaged in bottles (200 ml capacity) is expected to Rs. 2.26. If the volume of operation is increased beyond 1000 kg per day, the cost of manufacture per bottle is expected to come down further.

6.6 Majority of the soft drinks sold in the market have less than 10 per cent total solids. Ingredients present in these soft drinks are sugar, flavourings, colours, acids and carbondioxide. Soft drinks have become very popular and their growth in Indian market in recent years is phenomenal. The cost of each bottle of popular brand of soft drink (200-250 ml) vary between Rs. 3.00 to 3.50 in retail outlets. Since there is a tremendous consumer preference for soft drinks, if a nutritionally better variety of soft drinks is made available. It is hoped that this may be received well by the consuming public. This gap could easily be filled by directly acidified milk beverage. Milk based beverages have besides of milk fat and protein, other nutrients like minerals and vitamins derived from milk. Therefore, it is definitely a nutritionally superior beverage than ordinary carbonated soft drink. The technological process developed, is simple and could easily be adopted by any dairy factory or private entrepreneur for the production of directly acidified milk beverage.

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ANNEXURE I

EVALUATION CARD FOR HEDONIC RATING TEST FOR ACIDIFIED MILK BEVERAGE

Batch No.....   Date ......

Name of judge ....   Time ......

Please rate the samples for quality attributes according to the 9-point Hedonic scale given below:

1.	Liked extremely	9
2.	Liked very much	8
3.	Liked moderately	7
4.	Liked slightly	6
5.	Neither liked nor disliked	5
6.	Disliked slightly	4
7.	Disliked moderately	3
8.	Disliked very much	2
9.	Disliked extremely	1

Characteristics	Sample No.				
	1	2	3	4	5
Flavour					
Body and texture					
Colour and appearance					
Overall acceptability					

Remarks, if any

Sample No. 1

2

3

4

5

ANNEXURE - II

PROFORMA FOR CONSUMER RESPONSE OF ACIDIFIED MILK BEVERAGE

Please answer the following questions

1. Age: Under 16.......... 16 to 25 ....

   25 to 50.......... Over 50 ....

2. Sex: Male .... Female ....

3. Occupation and Designation ....

   ....

4. Do you like sour drinks?

   Extremely ....

   Moderately..........

   Slightly ....

   Do not like ....

5. Please place check mark (_/) to indicate your rating of the product:

Acceptable range	Non-acceptable range
10. Excellent........	5. Slightly undesirable....
9. Very good........	4. Definitely undesirable ......
8. Good	3. Unpleasant ....
7. Fair	2. Very unpleasant.......
6. Poor	1. Repulsive.............

6. Remarks, if any

Date